HYBRIDIZATION OF TWO SOUTHERN APPALACHIAN BLUEBERRY SPECIES WITH RABBITEYE AND SOUTHERN HIGHBUSH BLUEBERRY

By

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Abstract of Dissertation Presented to the Graduate School
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HYBRIDIZATION OF TWO SOUTHERN APPALACHIAN BLUEBERRY SPECIES WITH RABBITEYE AND SOUTHERN HIGHBUSH BLUEBERRY

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Vaccinium constablaei (*2n = 6x = 72*) and *V. simulatum* (*2n = 4x = 48*) are wild blueberry species native to the Appalachian mountains that might be used in breeding to improve rabbiteye (*V. ashei*, *2n = 6x = 72*) and highbush (*V. corymbosum*, *2n = 4x = 48*) blueberries in Florida. Greenhouse experiments showed that *V. ashei* x *V. constablaei*, *V. ashei* x F1 (*V. ashei* x *V. constablaei*), F1 (*V. ashei* x *V. constablaei*) x F1 (*V. ashei* x *V. constablaei*), *V. corymbosum* x *V. simulatum* and their reciprocal crosses were, on average, fertile and produced abundant progeny. However, *V. ashei* x *V. simulatum* and *V. corymbosum* x *V. constablaei* crosses produced small progeny populations. Self-

pollination decreased fertility in *V. ashei*, *V. constablaei* and their F1 hybrids compared to cross pollination, but F1 *V. ashei* x *V. constablaei* hybrids had higher self-fertility, on average, than *V. ashei* clones.

Compared to *V. ashei*, desirable characteristics observed in the *V. ashei* x *V. constablaei* F1 hybrids also included later flowering, shorter bloom-to-ripe period, larger corolla aperture, smaller distance between the stigma and anther pore, and better leafing during flowering. Field observations revealed that late flowering and short bloom-to-ripe interval were also characteristics of the *V. corymbosum* x *V. simulatum* F1 hybrids, which were as fertile as *V. corymbosum* x *V. corymbosum* F1 hybrids when open-pollinated. Fruit characteristics that predominated in *V. ashei* x *V. constablaei* and in *V. corymbosum* x *V. simulatum* seedlings included black color, high firmness, small and dry picking scar, and size intermediate between the two parents.

Pollen fertility was high in *V. ashei*, *V. constablaei*, their F1 hybrids, *V. corymbosum* and *V. simulatum*. *Vaccinium corymbosum* x *V. constablaei* F1 hybrids averaged lower pollen fertility and higher frequency of unreduced, stainable pollen compared to the other taxa studied.

Vaccinium constablaei appears promising as a species to cross with *V. ashei* to obtain plants that flower later and ripen earlier. Large, highly-fertile F1 populations can be generated, but much work would be required to obtain segregates that are well-adapted to north Florida.

CHAPTER 1
INTRODUCTION

The species: *V. constablaei* and *V. simulatum*

Vaccinium constablaei ($2n = 6x = 72$) is a blueberry species in the section Cyanococcus. This species was first studied cytologically in 1927, when Longley reported that a blueberry plant collected under the name *Vaccinium pallidum* from Pisgah Ridge, in the mountains of western North Carolina, was hexaploid (Longley, 1927). In 1944, six blueberry plants collected from Grandfather Mountain in western North Carolina were found to be hexaploid by Darrow et al. (1944). These authors indicated that the plants whose chromosomes they counted were of the same species as the plant studied earlier by Longley, but they classified their plants as *V. constablaei* rather than *V. pallidum*.

Brightwell et al. (1949) described *V. constablaei* as being native to the high mountains of western North Carolina, having light-blue fruit, with plants 1.5 to 2.1m tall and suckering over an area of 1.5 to 5m diameter or more. Camp (1945), however, had earlier described *V. constablaei* as a species from the mountain tops and upper slopes of western North Carolina and eastern Tennessee, whose plants ranged from 0.5 to 8m tall and varied from crown-forming with few stems to suckering, forming colonies several meters in diameter.

During the late 1970s and early 1980s, Paul Lyrene made several collection trips to the mountains of western North Carolina to obtain hexaploid plants of *V. constablaei* to cross with *V. ashei* cultivars. Although plants were collected at the locations where Camp had found *V. constablaei*, and the plants fit Camp's description of *V. constablaei*, Lyrene found that all the plants he had collected were tetraploid by chromosome count. Subsequently, Lyrene made another trip to western North Carolina, this time accompanied by Jim Ballington Jr. of North Carolina State University, who suggested that only the low-growing, highly colonial plants typical of the mountain balds were likely to be hexaploid. Plants of this growth habit were collected and found to be hexaploid by Lyrene. It now appears almost certain that Camp's description of *V. constablaei* included both hexaploid (the shorter-stature spreading form) and tetraploid (the taller, more crown-forming form) plants. In this dissertation, the name *V. constablaei* will be used only in conjunction with the low-growing, highly colonial plants collected from high-light locations on the mountain balds above 1800m in western North Carolina and eastern Tennessee. The much more widespread, tall-growing, crown-forming plants from the high mountains of western North Carolina will be called *V. simulatum*.

Another source of *V. constablaei* material for this research came from clones in the U. S. Department of Agriculture germplasm repository in Corvallis, Oregon. These clones were low-growing, highly colonial plants, which were collected in the mountains of western North Carolina about 1940, probably by Camp and Darrow. Meader took these clones to New Hampshire and later sent seed or clones to Maine, where Hepler

tested them for cultivar potential. When Hepler retired, about 10 clones were sent to the Corvallis blueberry germplasm repository.

Vaccinium simulatum (*2n = 4x = 48*) is a tetraploid blueberry species in the section Cyanococcus and is also native to the Appalachians mountains. It appears to be much more abundant and widespread than *V. constablaei*, occurring on intermediate and high elevations from northern Alabama and Georgia to Kentucky and West Virginia (Camp, 1945). It has the form of a short tree, with an average plant height up to 6m (Ballington, 1990) and has few canes per clone.

According to Ballington (1990) and Luby et al. (1991), it is difficult to differentiate *V. simulatum* from *V. corymbosum* based only on leaf and fruit morphology. The resemblance between the two species is so great that Vander Kloet considered *V. simulatum* a form of *V. corymbosum* (Vander Kloet, 1980; 1988). Vander Kloet also considered *V. constablaei* to be a race of *V. corymbosum*. Ballington et al. (1987), however, presented evidence based on differences in the percentage of anthocyanins, aglycones, and aglycone-sugars in the fruit of *V. constablaei*, *V. simulatum* and *V. corymbosum* that supports the view that the three taxa constitute different species, as suggested by Camp (1945), Galletta (1975), and Lyrene (1993). Small (1933) referred to *V. simulatum* as *Cyanococcus simulatum* and was the first to describe this species. Complete morphological descriptions of the plant are provided by Small (1933) and also Camp (1945).

Research Objectives

This research was conducted to determine whether it is feasible to cross *V. ashei* x *V. constablaei* and *V. corymbosum* x *V. simulatum* and to assess the potential value of the hybrids in breeding rabbiteye (*V. ashei*) and highbush (*V. corymbosum*) cultivars. Specific objectives were a) measuring the crossability of these species as determined by the fruit set, seed content of the fruit, and seed germination; b) determining plant and fruit characteristics of parents and F1 clones, with emphasis on berry weight, color, picking scar, firmness, development period, flowering and ripening time, leafing/flowering time, self-fruitfulness and fertility; c) comparing self-fertility of parents and F1 clones from *V. ashei* x *V. constablaei* crosses; d) characterizing the flower morphology of parents and F1 clones from *V. ashei* x *V. constablaei* crosses, as well as for *V. corymbosum* and *V. simulatum*. Characteristics that were examined included corolla length, corolla width, corolla aperture, style length and the distance from stigma to anther pore; e) determining pollen viability and presence of unreduced gametes in *V. ashei*, *V. constablaei*, *V. corymbosum*, *V. simulatum* and some of their interspecific hybrids; f) obtaining indications of the chromosome number of the *V. constablaei* and *V. simulatum* clones used in this research.

Significance of this Research for Florida

Many species of blueberries (genus *Vaccinium*) are native to the USA. They constitute an economically important crop extensively planted for their edible fruits. Two

major species have been cultivated in Florida, *V. ashei* (rabbiteye) and *V. corymbosum* (highbush). Florida had about 1,000 ha planted with blueberries in 1992 (Williamson and Lyrene, 1995), of which approximately 400 ha were planted with *V. ashei* (Spiers, 1990) and the remaining area with *V. corymbosum*. The area planted with both blueberry species is expected to increase in Florida as early-ripening varieties that have higher yields, more dependable production, and good fruit quality, especially for the fresh market, are released, enhancing market opportunities for growers. Currently, the estimated production is about 4,000 kg/ha/year.

Breeding cultivars for early ripening has been a major goal in the southeastern U.S. Early ripening would allow growers to take advantage of the high prices received for early-season fresh blueberries (Lyrene and Sherman, 1984). Some early ripening cultivars have been released. Among them, 'Sharpblue', 'Misty', and 'Star' stand out as highbush blueberry cultivars (Lyrene and Sherman, 1988) and 'Beckyblue', 'Bonita', 'Climax' and 'Aliceblue' as rabbiteye cultivars (Spiers, 1990; Lyrene and Crocker, 1991). All of them, however, are very susceptible to freeze damage to the flowers and fruit in late winter and early spring (Lyrene and Crocker, 1991). Open blueberry flowers may be killed by temperatures below -2°C, according to Lyrene and Sherman (1984).

One approach that has been considered in breeding late-flowering, early-ripening, blueberries for Florida has been the hybridization of Florida rabbiteye cultivars with *V. constablaei* and Florida highbush cultivars with *V. simulatum*. Both *V. constablaei* and *V. simulatum* are wild blueberry species native to the western North Carolina and eastern Tennessee mountains (Camp, 1945). Some of the desirable characteristics of these wild

blueberries are high-quality berries with excellent flavor and small seeds, late flowering associated with short bloom-to-ripening interval and leafing that precedes blooming (Lyrene and Sherman, 1984). Various workers have shown that it is possible to cross *V. ashei* with *V. constablaei* (Meader and Darrow, 1944; Brightwell et al., 1949; Darrow et al., 1952; Ballington et al., 1986). In addition, *V. corymbosum* can be crossed with *V. simulatum*, since both species are tetraploid.

Brightwell et al. (1949) and Ballington et al. (1986) reported that the progeny of *V. ashei* x *V. constablaei* crosses inherit the late-flowering, early-ripening characteristics that result in a short fruit development period. Another outstanding desirable quality present in the F1 seedlings is the small dry scar (Brightwell et al., 1949). Lyrene (1994a) mentioned that *V. constablaei* can be used to breed rabbiteye cultivars with improved flower morphology, since *V. ashei* x *V. constablaei* F1 hybrids have floral features more favorable for pollination by honeybees. Although undesirable qualities are also expressed in the hybrids, such as the small size and dark color of the berries and lack of vigor of most of the plants, both Brightwell et al. (1949) and Ballington et al. (1986) agreed that there is sufficient variability for most traits to permit selection within hybrid seedling populations.

Some work has already been done to evaluate *V. ashei* x *V. constablaei* progenies and to ascertain their potential for the development of commercial blueberry cultivars. The selection program for these hybrids has two goals, depending upon the climatic zone in which the cultivars are to be grown (Lyrene, 1993). For areas such as Florida, where rabbiteye blueberries are native and traditionally cultivated, the goal is to develop low-

chill hexaploid hybrids that ripen earlier and have higher fruit quality than the earliest-ripening rabbiteye cultivars. The cultivar Snowflake, developed through the University of Florida breeding program, is an example. 'Snowflake', which was produced by backcrossing a *V. constablaei* x *V. ashei* F1 hybrid created at North Carolina State University with a low-chill Florida *V. ashei* selection, ripens earlier than any other rabbiteye cultivar in Florida. For areas farther north, the goal is to select hybrids with more cold-hardiness and a higher chilling requirement than the current rabbiteye cultivars have. An example is the hybrid blueberry cultivar Little Giant, which was released in 1995 and was recommended for trial in blueberry growing areas of North Carolina, New Jersey, southern Michigan, Oregon, and Washington (Agricultural Research Service, 1995). This cultivar was selected in 1967 by A. D. Draper and originated from the cross *V. constablaei* x the rabbiteye selection T-65. 'Little Giant' has small, dark-blue berries that have a good flavor and a very small picking scar. It has good fruit qualities for the processed marked, but has not been tested in Florida. Another *V. ashei* x *V. constablaei* hybrid, developed in North Carolina and known as NC1827, has performed well in the mountains of western North Carolina according to Ballington, cited by Lyrene (1993).

Vaccinium simulatum appears to have some of the same desirable characteristics present in *V. constablaei*. Besides Ballington's report that *V. corymbosum* x *V. simulatum* crosses do not appear to be fully interfertile (Ballington, 1990), there are no other reports and no published data in this respect, which suggests that *V. simulatum* has not yet been much used in breeding highbush blueberries. *Vaccinium simulatum* is,

however, a potential source of late-flowering, early-ripening, and fruit qualities like small seeds, good texture, small and dry picking scar and unique flavor and aroma.

CHAPTER 2
CROSSING EXPERIMENTS

Introduction

Blueberries are typically cross-pollinated. Crossing the cultivated blueberries, *V. ashei* and *V. corymbosum*, with wild germplasm has been shown to be feasible and has attracted the attention of breeders for many years. Interspecific hybridization has been used to avoid inbreeding depression and to introduce genes with desirable characteristics into the cultivated gene pool (Lyrene and Sherman, 1984).

The interspecific cross *V. ashei* x *V. constablaei* was made by Brightwell et al. (1949) and Ballington et al. (1986) to combine in the F1 hybrids certain characteristics of *V. ashei*, such as high yield, light-blue fruit color, excellent fruit firmness, small picking scar, superior shelf life, adaptation to mechanical harvesting and tolerance to upland soils, with desirable characteristics of *V. constablaei*, which include late flowering, short development period of the fruit, high berry quality, unique flavor components, and cold hardiness (Lyrene and Sherman, 1984; Lyrene and Ballington, 1986; Ballington, 1990). Another hybrid combination of interest is cultivated *V. corymbosum* crossed with *V. simulatum*. It might be expected that these species could be crossed since both are in *Vaccinium* section Cyanococcus and both are tetraploid (*2n=4x=48*). Nevertheless, there is no information available in the literature regarding these crosses, except that Ballington (1990) mentioned that "they do not appear to be fully interfertile" (p. 59).

The present research had the goal of testing the crossability among *V. ashei*, *V. constablaei*, their F1 hybrids, *V. corymbosum* and *V. simulatum*, using cultivars and advanced selections available through the University of Florida breeding program. Another goal of the research was to compare the self-fertility of parents and F1 plants from *V. ashei* x *V. constablaei* crosses, since self-fertility is a desirable characteristic that could enhance yield in situations in which cross-pollination is reduced.

Note on Clone Names

Throughout the dissertation, clone numbers written without a letter prefix are FL numbers assigned by the University of Florida blueberry breeding program, e. g., 91-59 is FL 91-59.

Materials and Methods

Experiment 1

This experiment was based on crosses made in the greenhouse in 1991. Four types of crosses were tested: *V. ashei* x *V. ashei*, *V. ashei* x *V. constablaei*, *V. ashei* x *V. simulatum* and *V. corymbosum* x *V. constablaei*. In these crosses, *V. ashei* and *V. constablaei* were hexaploid, *V. simulatum* and *V. corymbosum* were tetraploid. The female parents used came from a field nursery in Gainesville, FL, and originated from the University of Florida blueberry breeding program. The selected plants were dug, potted and transferred to a greenhouse, where they were pruned in order to remove weak twigs and branches with no flower buds.

The pollination was done from February to April, 1991. Fully developed, unopened flowers were emasculated with forceps, and immediately pollinated using pollen from the male parent. To gather the pollen, flowers were rolled between the fingers in such a way that the pollen was shed onto the thumbnail. The pollen-laden thumbnail was then gently touched to the stigmas of the emasculated flowers until they were covered by pollen grains. At least 50 flowers were pollinated per cross. Most of the *V. constablaei* pollen used in these crosses came from *V. constablaei* selections maintained at the USDA National *Vaccinium* clonal repository in Corvallis, Oregon. The clones used included *V. constablaei* clones 4, 5, 6, 7, 8 and 11 from the repository. Dormant branches bearing flower buds were sent from the repository in January, 1991. The flowers were forced to develop by placing the branches in a commercial flower preservative solution. Pollen from two *V. constablaei* clones, NC 83-19-10 and NC 86-36-4, provided by Jim Ballington at North Carolina State University, was also used in some crosses. The *V. simulatum* pollen came from flowers produced on branches taken from Grandfather Mountain in western North Carolina in December, 1990. These plants were 2-4m tall in their native habitat. Pollen from eight Grandfather Mountain clones was used in the 1991 crosses, and also pollen from Vander Kloet 123877, a *V. simulatum* clone collected by Sam Vander Kloet. *Vaccinium ashei* pollen came from cultivars and advanced selections of the University of Florida blueberry breeding program.

Mature fruits were harvested from April to July and counted. Fruit set was calculated per cross as the percentage of the number of ripened fruits produced in relation to the number of pollinated flowers.

Experiment 2

In 1992, another set of crosses was made in the greenhouse including *V. ashei* x *V. ashei*, *V. ashei* x F1 (*V. ashei* x *V. constablaei*) hybrids, *V. ashei* x *V. constablaei*, *V. constablaei* x *V. ashei*, *V. corymbosum* x *V. simulatum* and *V. corymbosum* x *V. corymbosum*. The *V. constablaei* parents used in these crosses originated from open-pollinated seed collected from the *V. constablaei* field plantings at the National Clonal Repository for *Vaccinium* at Corvallis, Oregon. Seed was composited from several clones. The seedlings were grown in field nurseries in Gainesville, FL. Seedlings were selected at the time of flowering for *V. constablaei* characteristics (late flowering, uneven budbreak, decumbent growth habit) to eliminate any interspecific hybrids that might have arisen from the open-pollinated seed. The seedlings used in crosses were dug from the nursery in December, potted in peat moss, given two months of chilling in a refrigerator, and then placed in a greenhouse to induce flowering.

The *V. simulatum* pollen parents originated on Grandfather Mountain in western North Carolina. Open-pollinated seed was collected in the summer of 1990 from 2-4m tall plants found at elevations above 1500m. Seeds were germinated in a greenhouse and seedlings were grown at the University of Florida Horticultural Unit in Gainesville, FL. Seedlings with flower buds were dug from the field in December, 1991, potted in peat moss and chilled for 2 months in a refrigerator. Flowering was then induced in a greenhouse. The F1 (*V. ashei* x *V. constablaei*) clones used as pollen parents were NC 1827 and NC 1832 provided by J. Ballington, North Carolina State University.

Vaccinium ashei and *V. corymbosum* (highbush) parents originated from the University of Florida blueberry breeding program.

Pollination was carried out from February through June. Procedures were the same as described for experiment 1. Fruit set was determined by counting the number of fruits produced from April to July, and expressed as a percentage of the number of pollinated flowers.

Experiment 3

This experiment was carried out in the greenhouse in 1993 and consisted of 3 types of crosses: *V. ashei* x *V. ashei*, *V. ashei* x *V. constablaei* and *V. ashei* x *V. simulatum*. The number of crosses of each type was 9, 10 and 1, respectively (Table 2.1).

In January, 1993, *V. ashei* female parents were selected from a field nursery in Gainesville, FL. They were dug, potted, and transferred to a cold room with a temperature of about 7°C where the plants remained approximately 40 days to complete their chill requirement. The plants were 1.5 years old from softwood cuttings and were 80 to 150cm tall and strongly branched. On February 25, the plants were placed in a greenhouse where they were pruned to remove weak twigs and branches with no flower buds. Two plants were available per clone, and for most clones one plant was pollinated with pollen from a different *V. ashei* clone and the other with *V. constablaei* pollen. For the *V. ashei* clone 91-59, one plant was divided into 2 halves, each half being pollinated with a different *V. constablaei* clone, and the other plant received only *V. ashei* pollen. One plant of *V. ashei*, clone 90-217, was divided into 2 halves, one of which received *V.*

ashei pollen and the other *V. constablaei* pollen. The second plant was pollinated only with *V. simulatum* pollen.

Table 2.1 - Description of *V. ashei* x *V. ashei*, *V. ashei* x *V. constablaei* and *V. ashei* x *V. simulatum* crosses made in the greenhouse in 1993.

Cross	Seed parent[Z]	Pollen parent[Y]	Parental ploidy levels
1	89-186	T 339[X]	6 - 6
2	89-186	constablaei # 5	6 - 6
3	91-59	Chaucer[X]	6 - 6
4	91-59	constablaei # 8	6 - 6
5	91-59	constablaei # 13	6 - 6
6	91-283	Powderblue[X]	6 - 6
7	91-283	constablaei # 11	6 - 6
8	90-225	Brightwell[X]	6 - 6
9	90-225	constablaei # 14	6 - 6
10	91-287	constablaei # 16	6 - 6
11	91-287	79-15[X]	6 - 6
12	91-103	constablaei # 1	6 - 6
13	91-103	93-10[X]	6 - 6
14	87-321	L[X]	6 - 6
15	87-321	constablaei # 9	6 - 6
16	91-281	T105[X]	6 - 6
17	91-281	constablaei # 15	6 - 6
18	90-217	simulatum # 17	6 - 4
19	90-217	constablaei # 6	6 - 6
20	90-217	93-25[X]	6 - 6

[Z] All are *V. ashei*.
[Y] *V. constablaei* pollen parents 1, 5, 6, 8, 9, 11 and 16 as well as *V. simulatum* #17 are wild clones from Shining Rock Wilderness Area, near Asheville, NC; *V. constablaei* clones 13, 14 and 15 are wild plants from Roan Mountain, NC.
[X] *V. ashei* cultivar or advanced selection from the University of Florida blueberry breeding program.

Pollen from *V. ashei* came from cultivars and advanced selections of the University of Florida blueberry breeding program. Pollen from *V. constablaei* and *V. simulatum* came from flowers forced from dormant branches collected from plants growing wild in the mountains of western North Carolina. Twelve *V. constablaei* and

one *V. simulatum* clones came from the Shining Rock Wilderness Area, south of Asheville, North Carolina. Additionally, four *V. constablaei* clones came from Roan Mountain. The plants from Roan Mountain were measured in situ. They were highly colonial, with hundreds of canes per clone. The canes from each colony spread over an area 3-10m in diameter, and the height of the tallest canes ranged from 90 to 130cm. The branches were cut from plants selected for high vigor on December 23, 1992. They were placed into plastic bags with wet paper towels to maintain moisture, and were immediately put into an ice chest with ice (0°C) where they remained until January 4, 1993. Then, they were transferred to a cold room with a temperature of about 7°C. At the time they were originally collected, the branches had already received many hours of chilling in the mountains. The bags with the branches in the cold room were opened and checked every 2 weeks, and if necessary, the wet paper towels were replaced. On February 4, the branches were transferred to the greenhouse, their bases were recut and they were put in 4-liter plastic buckets containing 1.5 liters of a preservative solution per bucket. Once a week, their bases were recut and the preservative solution renewed.

In order to determine the best preservative solution to use, a flower preservation test was carried out during 3 weeks, beginning on January 15, 1993. Three types of solutions were compared: (a) water; (b) sucrose (5%) + ethanol (1%), and (c) sucrose (5%) + citric acid (5.93×10^{-4}M) + 8-hydroxy quinoline hemisulfate salt (5.93×10^{-4}M). Cut branches of *V. ashei* 83-63 were placed in buckets containing the solutions (depth 10cm) with 2 replications per treatment. The buckets with the branches were placed on a laboratory bench top where the temperature was about 20°C with subdued room lighting

from fluorescent lamps in the ceiling for about 8h a day. The solutions were changed and the stems recut once a week. The preservative solution selected was the one containing sucrose + citric acid + 8-hydroxy quinoline hemisulfate salt because in this treatment the flower buds showed the most vigorous development.

The pollinations for experiment 3 were made in March, 1993. Specific dates were recorded. General procedures were the same as described for experiment 1, and 100 flowers were pollinated per cross. Only 10 out of the 16 *V. constablaei* clones and 1 *V. simulatum* were used in this experiment. Some clones did not produce enough flowers on the cut branches, so they were not used. Ripening of the fruit produced by hand-pollination occurred from May through August. Harvest dates were recorded. Fruits were collected every 3 days, counted, and weighed. Fruits were then stored in paper bags at 7°C until harvest was complete for each cross. Seeds were counted from the first 30 berries that ripened from each cross. The remaining berries had their seeds extracted with a food blender. The seeds were washed several times with water to remove fruit residues, and were then dried on paper towels before being weighed. The dried seeds were then stored in coin envelopes at 5°C. A seed sample of 0.30g was weighed per cross, treated with a fungicide powder (Captan) and planted in small pots filled with peat in the greenhouse on December 3 in order to assess germination. The greenhouse temperature varied between 7°C and 25°C. The seeds were spread on the surface of the peat and kept wet by a mist irrigation system. After approximately 3 months the seedlings were counted.

Evaluations were as follows: fruit set, calculated as the percentage of fruits produced in relation to the number of pollinated flowers; fruit development period, which was the number of days between the first pollinated flower and the first harvested fruit; berry weight, expressed as the mean weight of the first 50 berries harvested; seeds per berry, being the average number of well-developed seeds in the first 30 berries; seed weight, the total weight of all seed from the cross; and number of seedlings per 100 pollinated flowers, estimated from the seed samples planted. To estimate the number of seedlings per 100 pollinated flowers, the number of seedlings obtained from the planted 0.30g of seed was used in conjunction with total seed weight from the cross to calculate how many seedlings would have been obtained had all seeds been planted.

Experiment 4

Another experiment was carried out in the greenhouse in 1993 using the methods of experiment 3 but involving four tetraploid *V. corymbosum* (highbush) clones as female parents, pollinated with pollen from *V. ashei* (6x), *V. constablaei* (6x) and *V. simulatum* (4x). Two plants were available for three of the clones that were used as females. For each of these clones, one plant received *V. ashei* pollen and the other *V. constablaei* pollen. Only one plant was available for the *V. corymbosum* clone 93-39. The branches of this plant were separated into 3 sectors, one being pollinated with *V. ashei*, another with *V. constablaei* and the third with *V. simulatum* pollen. A description of the crosses is presented in Table 2.2.

Pollen from *V. ashei* came from cultivars and advanced selections of the University of Florida blueberry breeding program. Pollen from *V. constablaei* and *V.*

simulatum came from flowers formed on the branches collected from native populations in the North Carolina mountains and forced in a greenhouse after chilling. The remaining procedures and evaluations were the same as already described for experiment 3.

Table 2.2 - Description of *V. corymbosum* (highbush) crosses with *V. ashei*, *V. constablaei* and *V. simulatum* in the greenhouse in 1993.

Cross	Seed parent[Z]	Pollen parent[Y]	Parental ploidy levels
1	93-39	constablaei # 14	4 - 6
2	93-39	simulatum # 17	4 - 4
3	93-39	79-15[X]	4 - 6
4	90-178	constablaei # 12	4 - 6
5	90-178	L[X]	4 - 6
6	90-182	Brightwell[X]	4 - 6
7	90-182	constablaei # 1	4 - 6
8	90-175	Powderblue[X]	4 - 6
9	90-175	constablaei # 7	4 - 6

[Z] All are *V. corymbosum*.
[Y] *V. constablaei* pollen parents 1 and 7 and *V. simulatum* #17 are native plants from Shining Rock Wilderness Area, near Asheville, NC; *V. constablaei* parents 12 and 14 are wild plants from Roan Mountain, NC.
[X] *V. ashei* cultivar or advanced selection from the University of Florida blueberry breeding program.

Experiment 5

Another experiment, conducted simultaneously with experiments 3 and 4 in 1993, was designed to study the female fertility of F1 (*V. ashei* x *V. constablaei*) hybrids when self pollinated or pollinated with pollen either from *V. ashei* or from a *V. ashei* x *V. constablaei* F1 hybrid from another family. Five F1 families of full-sibs were chosen for use in the experiment. *Vaccinium ashei* was the pollen parent in 11 crosses and F1 hybrids in 12 crosses; eight F1 clones were self pollinated (Table 2.3).

Table 2.3 - Description of crosses made in the greenhouse in 1993 among F1s between *V. ashei* and *V. constablaei*, also including selfs and pure lines of *V. ashei* as the pollen parent.

Cross	Seed parent		Pollen parent	Type of cross
	Source[z]	Clone		
1	(97)	93-94	(2) 93-107	F1 x F1
2	(97)	93-95	(107) 93-110	F1 x F1
3	(97)	93-95	(97) 93-95	F1 x Self
4	(97)	93-96	FL 87-321	F1 x Ash
5	(97)	93-97	Powderblue	F1 x Ash
6	(1)	93-98	NC 1832	F1 x F1
7	(1)	93-99	(3) 93-106	F1 x F1
8	(1)	93-99	(1) 93-99	F1 x Self
9	(1)	93-100	FL 87-273	F1 x Ash
10	(96)	93-101	(1) 93-98	F1 x F1
11	(96)	93-101	(96) 93-101	F1 x Self
12	(96)	93-102	NC 1827	F1 x F1
13	(96)	93-103	ash L	F1 x Ash
14	(96)	93-104	FL 83 -109	F1 x Ash
15	(3)	93-105	(107) 93-117	F1 x F1
16	(3)	93-106	FL 93-16	F1 x Ash
17	(107)	93-108	(2) 93-107	F1 x F1
18	(107)	93-108	(107) 93-108	F1 x Self
19	(107)	93-109	(2) 93-107	F1 x F1
20	(107)	93-109	(107) 93-109	F1 x Self
21	(107)	93-110	(97) 93-97	F1 x F1
22	(107)	93-110	(107) 93-110	F1 x Self
23	(107)	93-111	(3) 93-105	F1 x F1
24	(107)	93-112	(97) 93-96	F1 x F1
25	(107)	93-112	(107) 93-112	F1 x Self
26	(107)	93-113	Chaucer	F1 x Ash
27	(107)	93-114	Brightwell	F1 x Ash
28	(107)	93-115	FL 87-273	F1 x Ash
29	(107)	93-116	FL 93-84	F1 x Ash
30	(107)	93-117	FL 87-255	F1 x Ash
31	(107)	93-117	(107) 93-117	F1 x Self

[z] (97) Family of full-sibs of 85-97 giant ash x Corvallis constablaei 8.
(2) 91-2 ash x Corvallis constablaei 1 - one plant only used as male.
(1) Family of full-sibs of 85-97 ash x Corvallis constablaei 5.
(96) Family of full-sibs of 91-5 ash x Corvallis constablaei 8 + 10.
(3) Family of full-sibs of 85-109 ash x Corvallis constablaei 8.
(107) Family of full-sibs of 89-191 giant ash x constablaei NC 86-36-4.

The F1 seedlings were potted from field nurseries in December and processed as described for the previous experiments. Pollination took place in the greenhouse in March with 100 flowers pollinated per cross. From May to July, ripe fruits were harvested, counted and weighed. Evaluations were identical to experiment 3, except that the average number of seeds per berry was based on the first 10 berries that ripened.

Experiment 6

This experiment was conducted in the greenhouse in 1994 to compare the fertility of *V. ashei*, *V. constablaei* and their F1 hybrids as female parents when crossed with *V. ashei* and selfed. Several *V. simulatum* x *V. corymbosum* crosses were also included in this study. Overall, 15 *V. ashei* x *V. ashei,* 15 *V. ashei* selfs, 8 *V. constablaei* x *V. ashei,* 8 *V. constablaei* selfs, 18 F1 (*V. ashei* x *V. constablaei*) x *V. ashei*, 13 F1 selfs, and 4 *V. simulatum* x *V. corymbosum* crosses were made. These crosses are described in Table 2.4.

The *V. constablaei* and *V. simulatum* plants used in this study were grown from seed collected from the Shining Rock Wilderness Area and Grandfather Mountain, respectively. Two-year-old seedling plants of *V. constablaei* and *V. simulatum* were dug from a field nursery in Gainesville, planted in pots and put into a cold room at about 7°C on December 2, 1993. The *V. ashei* and F1 plants were dug from a field nursery, potted, and placed in the cold room on February 10, 1994. All plants were removed from the cold room on March 8 and transferred to the greenhouse. At this time, all but the *V. ashei* plants were sprayed with 2% oil to protect the flower buds from blueberry gall midge (*Dasineura oxycoccana*).

Table 2.4 - Description of experiment # 6 crosses in the greenhouse in 1994.

Seed parent			Pollen parent	
	(*V. ashei*)		(*V. ashei*)	(Selfed)[Z]
Cross	Source	Clones		
1		83-109	Powderblue	+
2		85-101	Brightwell	+
3		92-164	83-109	+
4		92-251	Brightblue	+
5		92-252	Brightblue	+
6		92-254	93-15	+
7		92-255	Tifblue	+
8		92-258	94-84	+
9		92-265	93-15	+
10		92-267	Tifblue	+
11		92-272	Brightwell	+
12		92-273	94-9	+
13		92-279	94-9	+
14		92-282	83-109	+
15		92-285	Powderblue	+
	F1[Y]		(*V. ashei*)	(Selfed)[Z]
Cross	Source[X]	Clones		
16	(100)	94-42	83-109	+
17	(100)	94-43	93-15	-
18	(100)	94-44	Brightwell	-
19	(100)	94-45	Powderblue	+
20	(98)	94-46	94-84	-
21	(98)	94-47	Powderblue	+
22	(98)	94-48	83-109	+
23	(98)	94-49	93-15	+
24	(101)	94-50	Brightblue	-
25	(101)	94-51	Tifblue	+
26	(101)	94-52	94-9	+
27	(101)	94-53	Tifblue	-
28	(107)	94-55	-	+
29	(107)	94-56	Brightblue	+
30	(107)	94-57	94-84	+
31	(95)	94-58	94-9	+
32	(95)	94-59	Brightwell	+
33	(95)	94-60	Brightblue	-
34	(95)	94-61	Tifblue	+

continuation (Table 2.4)

Seed parent			Pollen parent	
(*V. constablaei*)			(*V. ashei*)	(Selfed)[z]
Cross	Source[x]	Clones		
35	(126)	93-330	-	+
36	(126)	93-331	Tifblue	-
37	(126)	93-332	Powderblue	-
38	(126)	93-333	-	+
39	(126)	93-334	-	+
40	(126)	93-335	-	+
41	(126)	93-336	-	+
42	(126)	93-337	-	+
43	(126)	93-338	-	+
44	(126)	93-339	83-109	-
45	(126)	93-340	94-9	-
46	(126)	93-341	-	+
47	(126)	93-42	93-15	-
48	(126)	93-43	94-84	-
49	(126)	93-44	Brightwell	-
50	(126)	93-45	Brightblue	-
(*V. simulatum*)			(*V. corymbosum*)	(Selfed)[z]
Cross	Source[x]	Clones		
51	(121)	93-349	93-34	-
52	(121)	93-350	6-19	-
53	(121)	93-351	Reveille	-
54	(121)	94-41	93-34	-

[z] (+) Means that the pollination was done and (-) means that it was not done.

[y] F1 Corresponds to first generation hybrids between *V. ashei* x *V. constablaei*.

[x] (95) Family of full-sibs of 85-109 ash x Corvallis constablaei 8.

(98) Family of full-sibs of 89-178 ash x Corvallis constablaei 8.

(100) Family of full-sibs of 85-97 ash x Corvallis constablaei 5.

(101) Family of full-sibs of 91-2 ash x Corvallis constablaei 11.

(107) Family of full-sibs of 89-191 ash x constablaei NC 86-36-4.

(121) *V. simulatum* seedlings originated from a composite of open pollinated seeds from 30 plants from Grandfather Mountain.

(126) *V. constablaei* seedlings originated from a composite of open pollinated seeds from 100 clones from Shining Rock Wilderness Area.

In the greenhouse, plants were prepared for pollination by removing weak twigs and branches with no flower buds. On *V. ashei* and F1 plants with sufficient flower buds, branches were divided into 2 halves, one half to be selfed and the other to be pollinated with pollen from a different *V. ashei* clone. However, *V. constablaei* seedlings were too small to be divided. Therefore, eight plants were randomly chosen to be selfed and eight to receive *V. ashei* pollen.

Pollination was done in March following the steps already described. Eight distinct *V. ashei* cultivars and advanced selections were chosen as pollen sources in such a way that each pollen source was used to pollinate 2 *V. ashei* and 2 *V. constablaei* plants. Fifty flowers were pollinated per cross and pollination dates were recorded. *Vaccinium ashei* flowers not used in the pollinations were removed to avoid their production of parthenocarpic fruits. Parthenocarpy was not observed in *V. constablaei*, *V. simulatum* or F1 plants, so their excess flowers were not removed.

Ripening occurred from May through July. Fruits were harvested every 3 days, counted and weighed. Specific harvest dates were also recorded. Well-developed seeds were counted from the first 10 berries. Seeds were extracted from the remaining berries with a blender, placed on paper towels to dry, and then weighed and stored in small paper bags in a refrigerator. Germination was tested using a seed sample of 0.30g (circa 500 well-developed seeds) planted on October 28 in pots filled with peat as previously described.

Maximum and minimum temperatures were recorded daily in the greenhouse during flowering and ripening from 2 thermometers hanging under the shade of 2 plants

at opposite ends of the greenhouse, one close to the exhaust fan and the other near the evaporative cooling pad.

Crosses were assessed for fruit set, fruit development period expressed in days and in Growing Degree Hours (GDH), mean berry weight, seeds per berry, total seed weight and total number of seedlings. Procedures were the same as described for experiment 3, except that the number of seeds per berry was based on the average of the first 10 berries that ripened. The estimation of GDH followed method A described by Baskerville and Emin (1969), based on mean daily temperature, calculated as the average of the daily minimum and maximum temperatures. This method required a minimum threshold temperature below which there was no accumulation of GDH. The threshold selected for our model was 4.5°C, which had been used by Richardson et al. (1975) in peaches and later by Ballington et al. (1984) and Ballington et al. (1986) in blueberries. The minimum threshold temperature was then subtracted from the mean daily temperature and the result multiplied by 24h, giving an estimate of GDH for each day. According to the definition presented by Richardson et al. (1975), 1 GDH is equivalent to 1h at a temperature 1°C above the base temperature of 4.5°C. No upper threshold was set because there is no consistent information in the literature for blueberries. Although it is reasonable to assume that a maximum threshold exists, it should vary among species and even among cultivars and selections within species as demonstrated by Carlson and Hancock (1991). The average maximum temperatures in the greenhouse were 27.8°C in March (beginning on March 10), 30.7°C in April, 33.0°C in May, 35.2°C in June, and

35.9°C in July. The calculated daily GDHs were summed for each cross, from the day the first flower was pollinated until the day the first ripe fruit was harvested.

Other Crosses

Two *V. ashei* clones (87-321 and 91-59), which produced few fruits when crossed with another *V. ashei* in 1993, and the F1 (*V. ashei* x *V. constablaei*) hybrid 93-95, which showed high self-fertility in the same year, were again pollinated in 1994. Two plants of each *V. ashei* clone and the one plant of the F1 hybrid clone were saved and stored in the cold room on December 20, 1993. Plants were removed from the cold room and transferred to the greenhouse on February 9, 1994, for pollination.

One plant of each of the *V. ashei* clones was used to repeat the crosses which had previously failed (87-321 x *V. ashei* clone L and 91-59 x 'Chaucer'). The other plant of each clone was pollinated with 'Powderblue' as a control. The branches of the F1 hybrid plant were separated into 2 sectors; one was selfed again and the other was pollinated with 'Powderblue'. Pollinations were made as previously described. One hundred flowers were pollinated per cross and the remaining flowers of the *V. ashei* plants were removed to eliminate parthenocarpic fruit. Ripe fruits were harvested from April to June and evaluations were the same as described for experiment 3.

Statistical Analysis

Differences among types of crosses for each parameter measured in experiments 1, 2, 5 and 6 were tested by the analysis of variance using a completely randomized design with unequal number of replications. In experiments 3 and 4, differences among

types of crosses were tested for significance using a randomized complete block design. *Vaccinium constablaei* origin (Shining Rock vs. Roan Mountain) was compared in experiment 3 using a completely randomized design. Means were compared using Tukey's test if the analysis of variance showed significant treatment effects. Correlations between seed number and fruit weight, and seed number and fruit development period were calculated for *V. ashei* and F1 (*V. ashei* x *V. constablaei*) hybrids, including data from self-pollination as well as cross-pollination. Significance of these correlations was tested using the t-test.

Results and Discussion

Fertility measurements to compare interspecific and intraspecific crosses among *V. ashei*, *V. constablaei*, *V. corymbosum* and *V. simulatum* included fruit set, fruit development period (in days and Growing Degree Hours), berry weight, number of seeds/berry, total seed weight and total number of germinated seedlings.

Fruit Set

In 1991, *V. ashei* x *V. ashei* crosses had a mean fruit set of 51.2%, which was significantly higher than the mean fruit set of *V. ashei* x *V. constablaei*, *V. ashei* x *V. simulatum* and *V. corymbosum* (highbush) x *V. constablaei* types of crosses (Table 2.5). *Vaccinium ashei* x *V. constablaei* crosses had an unexpectedly low fruit set (18.5%), considering that both species belong to the section Cyanococcus and have identical chromosome numbers. The low fruit set may have been due to the fact that the pollen was collected several weeks before the crosses were made, and the *V. constablaei*

Table 2.5 - Fruit set on crosses made in the greenhouse in 1991.

Type of cross [z]	Parental ploidy levels	Number of crosses[y]	Mean fruit set (%)[x]	Sample standard deviation[w]	Rank of mean
AA x AA	6 - 6	40	51.2 a	19.9	1
AA x CC	6 - 6	19	18.5 b	18.3	2
AA x SS	6 - 4	9	7.0 b	10.5	4
HH x CC	4 - 6	2	16.2 b	21.4	3

[z] AA = *V. ashei*; CC = *V. constablaei*; HH = *V. corymbosum*; SS = *V. simulatum*.
[y] Fifty to 392 flowers pollinated per cross.
[x] Means within a column followed by different letters are significantly different at $P \leq 0.05$ using the Tukey test.
[w] Based on variance among means for individual crosses.

branches from which it was collected were small and highly dormant at the time forcing began. Alternatively, the low fruit set from *V. ashei* x *V. simulatum* and *V. corymbosum* x *V. constablaei* crosses was expected due to the variable ploidy levels in parents, presumably a barrier for species intercrossing.

Vaccinium ashei x *V. ashei* crosses made in 1992 had a mean fruit set of 56.3%, which was consistent with the result obtained from this type of cross the previous year (Table 2.6). In addition, no significant differences for fruit set were found among *V. ashei* x *V. ashei*, *V. ashei* x F1 (*V. ashei* x *V. constablaei*) hybrids, *V. constablaei* x *V. ashei*, *V. corymbosum* x *V. simulatum* and *V. corymbosum* x *V. corymbosum* crosses. All of them produced a reasonable high mean fruit set. In 1992, only the *V. ashei* x *V. constablaei* crosses differed significantly from the other species combinations, with a low mean fruit set of 28.9%, possibly due to low pollen viability. *Vaccinium constablaei* was much more effective in producing fruit when used as the female parent in crosses with *V. ashei*; in 1992, the five *V. constablaei* x *V. ashei* crosses averaged a fruit set of 61% (Table 2.6). The fact that *V. simulatum* had low fruit set when crossed with *V. ashei* in

Table 2.6 - Fruit set on crosses made in the greenhouse in 1992.

Type of cross [z]	Parental ploidy levels	Number of crosses[y]	Mean fruit set (%)[x]	Sample standard deviation[w]	Rank of mean
AA x AA	6 - 6	21	56.3 a	24.1	5
AA x AC	6 - 6	11	60.0 a	21.9	3
AA x CC	6 - 6	14	28.9 b	17.9	6
CC x AA	6 - 6	5	61.0 a	13.8	2
HH x SS	4 - 4	7	57.2 a	14.8	4
HH x HH	4 - 4	15	76.2 a	8.5	1

[z] AA = *V. ashei*; AC = F1 between *V. ashei* x *V. constablaei*; CC = *V. constablaei*; HH = *V. corymbosum*; SS = *V. simulatum*.

[y] Fifty to 500 flowers pollinated per cross.

[x] Means within a column followed by different letters are significantly different at $P \leq 0.05$ using the Tukey test.

[w] Based on variance among means for individual crosses.

1991 and high fruit set when crossed with *V. corymbosum* (highbush) in 1992 probably resulted from the ploidy level of the parents being the same in 1992.

Mean fruit set from *V. ashei* x *V. constablaei* crosses in 1993 (40.6%) did not differ significantly from the mean fruit set of *V. ashei* x *V. ashei* crosses (53.3%) (Table 2.7). The differences between these results and those in Tables 2.5 and 2.6, where *V. ashei* x *V. constablaei* fruit set averaged lower than fruit set from *V. ashei* x *V. ashei*, may have been due either to differences between years in the amount of parthenocarpic fruits produced by *V. ashei* or to the fact that different clones were used in the crosses of different years. Indeed, some of the *V. ashei* clones produced considerable numbers of parthenocarpic fruit. These could be recognized by several features: they had no seeds; they were smaller than the seedy fruit; they were the last to ripen; and the corolla, having not been removed during emasculation, remained attached to the apex of the fruit. The parthenocarpic berries were very difficult to harvest; the stem was so firmly attached to

Table 2.7 - Fertility measurements in crosses made in the greenhouse in 1993 with *V. ashei* as female parent and *V. ashei, V. constablaei* and *V. simulatum* as pollen parents.[z]

	V. ashei x *V. ashei*	*V. ashei* x *V. constablaei*	*V. ashei* x *V. simulatum*[y]
Number of crosses	9	10	1
Fruit set (%)	53.3	40.6	31.0
Fruit development period (d)	65.6	68.8	69.0
Berry weight (g)	1.77	1.49	1.47
Seeds/berry (#)	24.4	13.4	0.5
Seed wt/100 flowers (g)	1.12	0.53	0.01
Seedlings/100 flowers (#)	709	402	4

[z] The analysis of variance shows no significant differences between the means at $P \leq 0.05$.

[y] The *V. ashei* x *V. simulatum* cross was not included in the statistical analysis because of insufficient replication, but it is mentioned for comparison.

the fruit that the fruit skin tore when the berry was picked, causing a bad scar. This tendency of producing parthenocarpic fruit was also observed in *V. corymbosum* (highbush) clones but not in *V. constablaei*, *V. simulatum* or in F1 (*V. ashei* x *V. constablaei*) hybrid clones. Therefore, fruit set may have been increased somewhat by parthenocarpy when *V. ashei* and *V. corymbosum* clones were used as female parents. Fruit that ripened later and were clearly parthenocarpic were excluded from the counts. It is also presumed that *V. constablaei* pollen obtained in 1991 and 1992 had low viability at the time it was used for pollinations.

Another factor that could have influenced fruit set means was the compatibility of the clones used in the crosses. Fruit set varied from less than 10% to above 90% among the crosses for both *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei*. Nevertheless, 70.8% of the *V. ashei* x *V. ashei* crosses had 50% or higher fruit set while only 40.0% of the *V. ashei* x *V. constablaei* crosses fit in this same category (Table 2.8). Genotype-

Table 2.8 - Distribution of fruit set among the crosses made in the greenhouse from 1993 to 1995 involving the species *V. ashei*, *V. constablaei*, their F1 hybrids, *V. corymbosum* (highbush) and *V. simulatum*, also including selfs.

Type of cross[z]	Fruit set categories (%)										Total number of crosses
	0.0 to 9.9	10.0 to 19.9	20.0 to 29.9	30.0 to 39.9	40.0 to 49.9	50.0 to 59.9	60.0 to 69.9	70.0 to 79.9	80.0 to 89.9	90.0 or above	
AA x AA	1	2	0	2	2	0	0	4	4	9	24
AA x self	4	2	2	0	1	1	1	1	1	2	15
AA x CC	4	1	0	0	1	1	0	0	1	2	10
AA x SS	0	0	0	1	0	0	0	0	0	0	1
AC x AA	1	0	2	3	2	3	5	3	3	7	29
AC x AC	0	0	2	2	0	2	1	2	1	2	12
AC x self	6	3	1	1	1	3	2	1	1	2	21
CC x AA	0	0	0	2	0	2	2	2	0	0	8
CC x self	8	0	0	2	2	0	0	0	0	0	12
SS x HB	1	0	1	0	1	0	1	0	0	0	4
HB x AA	0	0	0	0	0	0	0	0	1	3	4
HB x CC	0	0	0	0	0	0	0	0	1	3	4
HB x SS	0	0	0	0	0	0	0	0	1	0	1

[z] AA=*V. ashei*; AC=F1 hybrid between *V. ashei* x *V. constablaei*; CC=*V. constablaei*; HB=*V. corymbosum*; SS=*V. simulatum*.

environment interactions were also present which could have affected the results. Two *V. ashei* x *V. ashei* crosses made in 1993, 87-321 x ash L and 91-59 x Chaucer (Table 2.9) gave unexpected low fruit set. They were repeated in 1994, and the two seed parents were pollinated with other *V. ashei* clones, to study the possibility of specific *V. ashei* x *V. ashei* cross incompatibilities. The results (Table 2.9) indicated that all the crosses were highly compatible in the second year in terms of fruit set, although the number of seedlings per pollinated flower was low for one of the crosses that had low success in 1993. The results from these repeated crosses indicated a year x cross interaction in fruit set.

Mean fruit set for *V. ashei* x *V. ashei* crosses is well documented in the literature, with reported averages ranging from 47.0 to 69.2% (Meader and Darrow, 1944; El-Agamy et al., 1981; Garvey and Lyrene, 1987; Lyrene, 1988; Lyrene, 1990; Gupton and Spiers, 1994). Nevertheless, variation in fruit set for this type of cross is much larger when crosses are considered individually, and can go from zero to 95.1%, depending on the particular parental combination used (El-Agamy et al., 1981; Garvey and Lyrene,1987; Lyrene, 1988 ; Payne et al., 1989; Lyrene, 1990). Meader and Darrow (1944) reported 2 *V. ashei* x *V. constablaei* crosses with 52.8% and 57.1% fruit set, which is above the mean fruit set obtained for this type of cross in experiments 1, 2 and 3.

The *V. ashei* x *V. simulatum* cross made in 1993 (Table 2.7) had a low fruit set. This concurs with the results of previous crosses and may have been due in part to the fact that *V. simulatum* is tetraploid. It has also been shown that *V. simulatum* gives high fruit set when crossed with tetraploid *V. corymbosum* (Tables 2.6 and 2.10).

Table 2.9 - Fertility measurements of 2 *V. ashei* clones, 87-321 and 91-59, pollinated by other *V. ashei* in the greenhouse in 1993 and 1994.

	1993 crosses		1994 crosses			
Fertility feature	87-321 x ash L	91-59 x Chaucer	87-321 x ash L	87-321 x Powderblue	91-59 x Chaucer	91-59 x Powderblue
Fruit set (%)	3.0	16.0	94.0	97.0	81.0	86.0
Fruit development period (d)	72.0	68.0	55.0	55.0	67.0	61.0
Berry weight (g)	1.18	1.36	2.22	2.20	2.29	2.93
Seeds/berry (#)	29.5	2.0	15.6	28.9	5.0	15.7
Seed wt/100 flowers (g)	0.034	0.044	1.431	1.652	0.147	0.984
Seedlings/100 flowers (#)	29	30	968	1327	208	879

Vaccinium corymbosum (highbush), when used as a female parent with either *V. ashei* or *V. constablaei*, gave a high fruit set in all crosses made in 1993 (Tables 2.8 and 2.10), despite the different ploidy levels. This result differed from that obtained from the *V. corymbosum* x *V. constablaei* crosses in 1991, in which fruit set was low. The 1993 crosses produced many non-viable seeds, which may have stimulated fruit production. There was also a strong parthenocarpic tendency in the *V. corymbosum* (highbush) plants used in 1993. These observations suggest that fruit set is not a good parameter to assess crossing success in blueberry. Data from Lyrene (1988) and Gupton and Spiers (1994) also indicate that tetraploid *V. corymbosum* can give high fruit set after pollination by *V. ashei*. According to Lyrene's research, tetraploid *V. corymbosum* x hexaploid *V. ashei* crosses averaged 76.3% for fruit set, with individual crosses ranging from 62.9 to 94.8%. This result was about the same as for tetraploid *V. corymbosum* intraspecific crosses, which averaged 73.9% and ranged from 63.6 to 81.0%. Furthermore, Gupton and Spiers

Table 2.10 - Fertility measurements in crosses made in the greenhouse in 1993 with *V. corymbosum* (highbush) as female parent and *V. ashei*, *V. constablaei* and *V. simulatum* as pollen parents.

	HB x *V. ashei*	HB x *V. constablaei*	HB x *V. simulatum*[Y]
Number of crosses	4	4	1
Fruit set (%)	93.0[Z]	93.5	82.0
Fruit development period(d)	50.8	50.0	55.0
Berry weight (g)	1.56	1.56	1.71
Seeds/berry (#)	24.4 a	11.8 b	28.3
Seed wt/100 flowers (g)	1.01	0.62	1.02
Seedlings/100 flowers (#)	93	20	1638

[Z] Means within a row followed by different letters are significantly different at $P \leq 0.05$ according to the analysis of variance.

[Y] The *V. corymbosum* x *V. simulatum* cross was not included in the statistical analysis because of insufficient replication, but is given for comparison.

obtained 72.0% fruit set by pollinating southern highbush flowers with *V. ashei* pollen, which is similar to the 71.0% they obtained from southern highbush intraspecific pollination and the 68.0% from *V. ashei* x *V. ashei* crosses. Mean fruit set of F1 (*V. ashei* x *V. constablaei*) x *V. ashei* crosses did not differ significantly from F1 x F1 crosses, but both types differed from F1 selfings in 1993 (Table 2.11). Nevertheless, fruit set in F1 x *V. ashei* crosses compared to F1 selfings was not statistically different in 1994 because some F1 plants showed a high level of fruit set when selfed (Tables 2.8 and 2.12). Lyrene (1990) reported 57.9% as the mean fruit set from 10 F1 (*V. ashei* x *V. constablaei*) x *V. ashei* crosses, which is very close to the 51.5% mean fruit set observed in the 1993 crosses.

Vaccinium ashei x *V. ashei* and F1 x *V. ashei* crosses had the highest fruit set (82.4 and 71.7%, respectively) in 1994, and did not differ from each other in this characteristic (Table 2.12). *Vaccinium constablaei* pollinated by *V. ashei* also provided a good fruit set (55.5%), somewhat lower than the fruit set of the two previously mentioned crosses but not statistically different. These results agree with the data presented in Tables

Table 2.11 - Fertility measurements in crosses made in the greenhouse in 1993 with F1 hybrids between *V. ashei* and *V. constablaei* as female parents and *V. ashei*, F1 hybrids (*V. ashei* x *V. constablaei*) and selfs as pollen parents.

	F1 x *V. ashei*	F1 x F1	F1 x self
Number of crosses	11	12	8
Fruit set (%)	51.5 a[z]	60.2 a	23.8 b
Fruit development period (d)	49.0 a	49.4 a	56.6 b
Berry weight (g)	1.05 a	0.96 a	0.59 b
Seeds/berry (#)	28.3 a	29.1 a	6.8 b
Seed wt/100 flowers (g)	0.76 ab	0.99 a	0.24 b
Seedlings/100 flowers (#)	1039 a	1422 a	161 b

[z] Means within a row followed by different letters are significantly different at $P \leq 0.05$ using the Tukey-test.

Table 2.12 - Fertility measurements in crosses made in the greenhouse in 1994 where *V. ashei*, *V. constablaei* and their F1 hybrids were crossed as female parents with *V. ashei* as pollen parent and/or selfed, and *V. simulatum* as female parent was crossed with *V. corymbosum* (highbush).

	V. ashei x *V. ashei*	*V. ashei* x self	F1 x *V. ashei*	F1 x self	*V. constablaei* x *V. ashei*	*V. constablaei* x self	*V. simulatum* x highbush
Total number of crosses	15	15	18	13	8	8	4
Fruit set (%)[v]	82.4 a[z]	38.7 bc	71.7 ab	45.8 ab	55.5 ab	6.2 c	32.2 bc
Number of crosses w/fruit[x]	15	12	18	10	8	2	3
Fruit set (%)[v]	82.4 a	48.3 ab	71.7 a	59.6 ab	55.5 ab	25.0 b	42.9 ab
Fruit development period (d)[v]	63.3 ab	76.2 a	49.0 c	52.0 bc	42.9 c	49.0 c	47.7 c
Fruit development (GDH)[v]	29112 ab	35560 a	23331 bc	24826 bc	20700 c	24101 bc	23319 bc
Berry weight (g)[v]	1.93 a	1.27 b	0.82 bc	0.72 bcd	0.42 cd	0.20 d	0.24 d
Seeds/berry (#)[v]	29.6 ab	2.5 c	33.4 a	10.6 c	40.4 a	13.2 bc	13.6 bc
Seed wt/50 flowers (mg)[v]	782 a	35 c	595 ab	252 bc	375 abc	100 c	124 bc
Seedlings/50 flowers (#)[v]	895 ab	45 c	1022 a	234 bc	852 ab	250 abc	125 bc

[z] Means within a row followed by different letters are significantly different at $P \leq 0.05$ using the Tukey-test.

[y] Mean calculated based on all crosses, including those that produced no fruit.

[x] Number of crosses that produced one or more fruits.

[v] Based on crosses that produced fruit.

2.5 and 2.6, where *V. constablaei* tended to average higher fruit set when used as the female parent (55.5% in Table 2.12) than as the male parent (40.6% in Table 2.7). Meader and Darrow (1944)reported 42.0% fruit set for a *V. constablaei* x *V. ashei* cross, which is within the range observed in the present study (Table 2.8). *Vaccinium simulatum* x *V. corymbosum* (highbush) crosses had a lower mean fruit set than *V. constablaei* x *V. ashei*, but were not significantly different.

Selfing caused a drastic reduction in the mean fruit set of *V. ashei*, *V. constablaei* and their F1 hybrids (Tables 2.11 and 2.12). Nevertheless, the reduction in fruit set caused by selfing was less severe in the F1 hybrids than in *V. ashei* and *V. constablaei.* Mean fruit set in selfed *V. ashei* clones ranged from 15.0 to 40.0% according to reports from the literature (Meader and Darrow, 1944; El-Agamy et al., 1981; Garvey and Lyrene, 1987; Gupton and Spiers, 1994). Once more, fruit set appeared not to be the best indicator of the fertility of a cross, because several *V. ashei* and F1 clones showed a fruit set higher than 80.0% when selfed (Table 2.8), despite having a large decrease in seed production and viability compared to compatible outcrosses. Some F1 clones seem to be more self-fertile than others. Self-fruitfulness in these F1 clones appeared to be relatively stable; one clone, when selfed for two consecutive years, showed consistent high levels of self-fruitfulness, with a fruit set of about 80.0% (Table 2.13). However, in both years it yielded far more seedlings per pollinated flower when crossed than when selfed.

The effect of *V. constablaei* origin on fruit set was tested, and there was no significant difference between Roan Mt. and Shining Rock Wilderness Area (Table 2.14). *Vaccinium constablaei* pollen from Shining Rock Wilderness Area tended to give higher

Table 2.13 - Fertility measurements in crosses made in the greenhouse in 1993 and 1994, where a self fertile *V. ashei* x *V. constablaei* F1 hybrid (93-95) was selfed and cross pollinated with another F1 hybrid and a *V. ashei* clone.

	1993 crosses		1994 crosses	
Fertility feature	(97) 93-95[Z] x F1 93-110[Y]	(97) 93-95 x Selfed	(97) 93-95 x Powderblue	(97) 93-95 x Selfed
Fruit set (%)	80.0	80.0	87.0	83.0
Fruit development period (d)	50.0	52.0	49.0	49.0
Berry weight (g)	1.15	0.89	1.20	1.03
Seeds/berry (#)	38.8	12.4	55.7	17.4
Seed wt/100 flowers (g)	1.989	1.200	2.695	1.672
Seedlings/100 flowers (#)	2651	784	3917	914

[Z] F1 hybrid line from the cross *V. ashei* FL 85-97 x *V. constablaei* Corvallis 8.
[Y] F1 hybrid line from the cross *V. ashei* FL 89-191 x *V. constablaei* NC 86-36-4.

Table 2.14 - Fertility measurements of *V. ashei* after pollination with *V. constablaei* pollen from two sources.

	V. constablaei origin[Z]	
Trait	Roan Mt	Shining Rock
Number of crosses	3	7
Fruit set (%)	24.3	47.6
Fruit development period (d)	73.0	67.0
Berry weight (g)	1.28	1.59
Seeds/berry (#)	5.7	16.8
Seed wt/100 flowers (g)	0.11	0.71
Seedlings /100 flowers (#)	108	528

[Z] The analysis of variance does not show significant differences for the means at $P \leq 0.05$.

values for all fertility parameters measured, but firm conclusions can not be drawn because the replication number was small and there was considerable variation within both populations.

Fruit Development Period

The period required for the blueberry fruits to fully develop and ripen on the seed parent was about the same for *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* crosses in

1993, with no statistically significant difference between them (Table 2.7). Non-significant results were also obtained for this trait when *V. corymbosum* (highbush) x *V. ashei* was compared to *V. corymbosum* x *V. constablaei* crosses (Table 2.10). F1 (*V. ashei* x *V. constablaei*) x *V. ashei* crosses did not differ significantly in fruit development period from F1 x F1 crosses (Table 2.11) made in the same year. These results suggest that the fruit development period in these crosses was determined by the clone bearing the fruit, and was less influenced by the pollen source. As a female parent, *V. ashei* had a longer fruit development period, about 65 days, than *V. corymbosum* (highbush) and *V. ashei* x *V. constablaei* F1s, in which the fruit development period was 50 and 49 days, respectively. Gupton and Spiers (1994) mentioned that berries from southern highbush pollinated with *V. ashei* took 58 days to develop and ripen, which was not significantly different from the 55 days needed by berries originated from southern highbush intraspecific pollination. By contrast, berries originated from *V. ashei* x southern highbush crosses required 100 days to ripen in the Gupton and Spiers study, demonstrating the primary influence of the female parent in the length of the fruit development period.

Crosses made in 1994 demonstrated the short flowering-to-ripening interval that was a principal reason for crossing *V. constablaei* with cultivated *V. ashei*. *Vaccinium ashei*, *V. constablaei* and F1 hybrid clones presented distinct fruit development periods, despite the fact that the same pollen sources were used to pollinate them all (Table 2.12). Fruits from *V. ashei* clones needed 63 days on average to ripen in the greenhouse. On the other hand, *V. constablaei* lines had a much shorter fruit development period, about

43 days, a statistically significant difference. The average fruit development period of the F1 hybrids, 49 days, was intermediate between that of the parents, but closer to *V. constablaei*. The F1 hybrids differed significantly from *V. ashei* but not from *V. constablaei*. Fruit development period in the *V. simulatum* clones was also short and did not differ from the *V. constablaei* and F1 clones. Meader and Darrow (1944) assessed the average number of days to berry maturity in two *V. ashei* x *V. constablaei* and one *V. constablaei* x *V. ashei* crosses, and they reported that the period of time required to berry maturity was much longer when *V. ashei* was used as the female parent (86.0 and 105.8 days) than when *V. constablaei* received pollen from *V. ashei* (49.3 days). Our data are consistent with these earlier observations.

Despite the evidence demonstrating this maternal effect, it has been shown that pollen source can affect the fruit development period in some blueberry crosses. El-Agamy et al. (1979) reported that fruit development time may vary for a particular female depending on the pollen source. Gupton and Spiers (1994) observed this effect, which they called 'xenia', in *V. ashei* and in southern highbush blueberries. The effect of pollen source on the fruit development period, which they observed, may have resulted because some pollen sources caused more seeds to form in the berries than other pollen sources. It is known in blueberry that high seed number per berry reduces the fruit development period. Gupton and Spiers noted a tendency for pollen from late-ripening cultivars to produce a longer fruit development period than pollen from early ripening cultivars. The effect of pollen source on fruit development period may have contributed to the variation within each type of cross, but was mostly evident when clones were selfed. Berries from

selfed flowers usually had a longer fruit development period than berries from cross-pollinated flowers, although the difference was not always significant. The tendency for selfing to delay fruit ripening was observed in *V. ashei* as well as in *V. constablaei* and F1 clones (Tables 2.11 and 2.12) and it was reported in *V. ashei* by Meader and Darrow (1944), Garvey and Lyrene (1987) and Gupton and Spiers (1994). The tendency for selfed plants to produce fewer seeds per berry may have been partially responsible for their longer fruit development period. This assumption is based on observations that, within crosses, blueberry fruits that ripened late tended to have fewer seeds than the first ripened ones (Moore et al., 1972). In fact, Gorchov (1985) attributed the asynchrony of ripening date of *V. corymbosum* fruits to variability in seed number, which was significantly correlated with the fruit development period in his study. Significant negative correlations ($P \leq 0.05$) of -0.51 and -0.39 between the number of well-developed seeds and fruit development period were obtained for *V. ashei* and F1 (*V. ashei* x *V. constablaei*) hybrids, respectively, in our study.

The fruit development period assessed as Growing Degree Hours (GDH) showed similar tendencies as when expressed in days. Again, the GDH requirement was determined in part by the female parent. *Vaccinium ashei* fruits required more GDH to ripen than *V. constablaei*, *V. simulatum* and F1 hybrid fruits, which did not differ significantly among themselves. Selfing tended to increase the GDH needed. Little information is available in the literature about GDH requirements for ripening blueberries. The data of Ballington et al. (1986) indicated that *V. ashei* has a high GDH requirement. F1 seedlings from a *V. ashei* x *V. ashei* cross in their study needed an

average of 37400 GDH to go from 50% flowering to 50% ripening, and F1 seedlings from 5 different *V. ashei* x *V. constablaei* crosses had a lower requirement, which varied from 19600 to 28700 GDH. Furthermore, two *V. ashei* populations from Florida evaluated for ripening by Ballington et al. (1984) in North Carolina also showed a high requirement of GDH, which varied from 39300 to 55100.

Vaccinium constablaei origin did not significantly affect the fruit development period, although pollen used from Shining Rock plants tended to make it slightly shorter (Table 2.14).

Berry Weight

Mean berry weight was not significantly different between *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* crosses, although it tended to be slightly lower in the second type of cross (Table 2.7). This could be related to the lower seed content of the fruits resulting from the interspecific cross, since Eaton (1967), Morrow (1943), Moore et al. (1972), Kushima and Austin (1979) and Gorchov (1985) demonstrated that some highbush and *V. ashei* blueberry cultivars show a significant positive correlation between fruit size and seed number. Previous work also mentioned that this relationship is not observed for all cultivars. In our study, *V. corymbosum* (highbush) x *V. ashei* gave the same berry weight as *V. corymbosum* x *V. constablaei* crosses, even though the former averaged more than twice as many seeds per berry (Table 2.10). *Vaccinium corymbosum* (highbush) x *V. simulatum* was a highly compatible cross, with both large fruit and a large number of well-developed seeds per berry. Gupton and Spiers (1994) reported that southern highbush pollinated with *V. ashei* pollen resulted in only a slightly lower berry

weight compared to intraspecific southern highbush pollination, although it gave fewer seeds. Our results agree with their report.

There was no apparent effect of fertility on berry weight when F1 (*V. ashei* x *V. constablaei*) hybrids were either crossed with another F1 or with *V. ashei*, although F1 x F1 crosses appear to be slightly more compatible (Table 2.11). On the other hand, a significant decrease in berry weight was noticed after selfing F1 clones in 1993. This effect of selfing on berry size was less severe in 1994. Berry weight was not significantly different for F1 clones that were selfed and others that were cross pollinated by *V. ashei* (Table 2.12). The F1 hybrid clones seemed to be more tolerant to selfing than *V. ashei* clones, in which selfing caused a significant reduction in fruit weight. According to Hellman and Moore (1983), there is a trend toward reduced berry weight with selfing but considerable variation exists among *V. ashei* cultivars in their response. This may be the reason El-Agamy et al. (1979) observed only a slight reduction in average berry weight of *V. ashei* clones after selfing compared to outcross pollination, contrary to our results. Larger reductions in berry weight for *V. ashei* were, however, reported by Meader and Darrow (1944), Garvey and Lyrene (1987), Payne et al. (1989), and Gupton and Spiers (1994). Mean berry weight was also reduced in *V. constablaei* after selfing. Reductions in berry weight due to selfing may be partially related to a decrease in the number of well-developed seeds compared to the higher seed content usually observed in berries produced from cross-pollinated flowers. Significant positive correlations ($P \leq 0.05$) of 0.61 and 0.42 between the number of well-developed seeds and berry weight of fruits of

V. ashei and F1 (*V. ashei* x *V. constablaei*) hybrids, respectively, were obtained in this study.

The berries of *V. ashei* were much larger than those of *V. constablaei,* and the F1s typically were intermediate in size (Table 2.12). This indicated that berry weight is largely inherent to the female parent, considering that the *V. ashei* pollen donors were the same for *V. ashei,* F1 and *V. constablaei* females in 1994. It is also important to emphasize that the *V. ashei* females used in the crosses came from the blueberry breeding program of the University of Florida and had been selected for large berries. Native populations of *V. ashei* would have much smaller berries (Garvey and Lyrene, 1987) but probably still somewhat larger than those of *V. constablaei. Vaccinium simulatum* x *V. corymbosum* (highbush) crosses also produced small fruits on the *V. simulatum* parent, not significantly different in size from *V. constablaei* fruits.

Berry weight did not statistically differ as a result of the origin of the *V. constablaei* pollinizer (Table 2.14). Berries were slightly larger where *V. constablaei* from Shining Rock Wilderness Area were used as male parents, but this could have been due to factors other than pollen source.

Seeds per Berry

The average number of seeds per berry in *V. ashei* x *V. ashei* crosses compared to *V. ashei* x *V. constablaei* ones was not significantly different (Table 2.7). By contrast, *V. corymbosum* (highbush) x *V. ashei* produced significantly more seeds per berry than *V. corymbosum* x *V. constablaei* (Table 2.10). Whether the female was highbush or *V. ashei,* the crosses produced less seed with *V. constablaei* pollen than with *V. ashei* pollen.

Along with many normal-looking seeds, the *V. corymbosum* x *V. ashei* and the *V. corymbosum* x *V. constablaei* crosses, but not the other crosses, produced many seeds that were just empty shells. A first indication came during the extraction from the berries, when many seeds floated in the water used to clean them. Later, it became evident that most seeds from these crosses were not viable, since they germinated poorly. These observations suggest that the number of seeds per berry is not a good parameter to assess fertility in some crosses. The *V. simulatum* clone brought from the North Carolina mountains for use as a pollen source caused the production of a high number of viable seeds per berry when crossed onto the tetraploid *V. corymbosum* but not when crossed with *V. ashei*, which is hexaploid. The difference in the average number of developed seeds per berry was remarkable, indicating that the *V. simulatum* clone was probably tetraploid.

F1 hybrids pollinated with *V. ashei* and F1 x F1 crosses produced a fairly high number of seeds per berry, indicating that the F1 hybrids are quite fertile and that the crosses are highly compatible. There were no significant differences in fertility between the two types of crosses. Both, however, differed from F1 selfings, in which the number of seeds per berry was severely reduced in 1993 (Table 2.11). Selfing was again detrimental to the fertility of F1, *V. ashei*, and *V. constablaei* clones in 1994, causing a significant reduction in the average number of seeds per berry (Table 2.12). *Vaccinium ashei*, however, tended to be more drastically affected than F1 and *V. constablaei* clones. Compared to results obtained after self pollination, *V. ashei* , *V. constablaei* and the F1 hybrids all produced more seeds per berry when they were cross pollinated using *V. ashei*

pollen. The three types of females did not show significant differences in this characteristic. *Vaccinium constablaei* female parents, however, tended to produce smaller seeds and more seeds per berry than *V. ashei*. F1 hybrids were intermediate. Small seed is a desirable feature of *V. constablaei* to be transferred into *V. ashei* commercial cultivars. *Vaccinium simulatum* x *V. corymbosum* crosses had a low number of seeds per berry on average.

Much information about seed number per berry in *V. ashei* intraspecific crosses is provided in the literature, which reports a large variation among crosses. There is general agreement that selfing reduces seed content (Meader and Darrow, 1944; Kushima and Austin, 1979; El-Agamy et al., 1981; Hellman and Moore, 1983; Garvey and Lyrene, 1987; Payne et al., 1989; Gupton and Spiers, 1994).

Significant differences in fertility were observed when *V. constablaei* was used as male or female parent in crosses with *V. ashei*. While *V. ashei* x *V. constablaei* crosses averaged 13.4 developed seeds per berry (Table 2.7), *V. constablaei* x *V. ashei* crosses averaged much higher, about 40.4 developed seeds per berry (Table 2.12). Similar results were found by Stushnoff and Palser (1969), who suggested that there might be some incompatibility between *V. ashei* stigmatic and/or stylar tissues and *V. constablaei* pollen tubes. This incompatibility would cause difficulties with *V. constablaei* pollen tube growth, and is non-existent when *V. constablaei*, as the female parent, is pollinated with *V. ashei* pollen. Another possible explanation is that *V. constablaei* flowers have shorter styles than *V. ashei* flowers, which would favor more pollen tubes to reach the ovary, consequently fertilizing more eggs. Based on El-Agamy et al. (1980), it is not a

gametophytic self-incompatible system, however, but post-zygotic abortion that occurs in *V. ashei* blueberries.

The number of seeds produced when *V. ashei* was pollinated by *V. constablaei* was influenced by *V. constablaei* origin; Roan Mt. *V. constablaei* induced only a third as many seeds as *V. constablaei* from Shining Rock Wilderness Area (Table 2.14). These differences were not statistically significant, however, and the phenomenon needs further study.

Total Seed Weight

The total seed weight originated per 100 pollinated flowers was not significantly different between *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* crosses (Table 2.7). *Vaccinium corymbosum* (highbush) x *V. ashei* did not differ significantly from *V. corymbosum* x *V. constablaei* in this parameter (Table 2.10). Whether the female parent was *V. corymbosum* or *V. ashei*, *V. constablaei* as male parent tended to induce a lower total seed weight than *V. ashei*, following the same tendency observed for the number of seeds per berry. Number of seeds per berry was apparently the major factor in determining the differences in the total seed weight in these crosses, although total seed weight also depends on seed size. A remarkable difference was again observed between *V. ashei* and *V. corymbosum* as female parents when both received pollen from the *V. simulatum* clone brought from the North Carolina mountains. The abundant seed production in the cross involving *V. corymbosum* compared to the one with *V. ashei*, which almost failed to produce seeds, probably indicates that *V. simulatum* is tetraploid, as discussed earlier.

F1 (*V. ashei* x *V. constablaei*) hybrids had a slightly lower total seed weight when pollinated with *V. ashei* pollen compared to when they were pollinated with pollen from other F1 hybrids in 1993 (Table 2.11). In the same year, F1 x *V. ashei* crosses did not differ significantly from F1 selfings, although seed production was somewhat reduced by selfing. A similar result was obtained in 1994 (Table 2.12). In both years, however, the number of fully-developed seeds was significantly less in F1 selfed clones compared to when outcross pollination took place. On the average, *V. constablaei* also experienced decreased total seed weight when selfed, but *V. ashei* was by far the most severely affected. *Vaccinium ashei*, *V. constablaei* and F1 clones produced normal seedy fruits when outcrossed with *V. ashei*, showing no significant differences in the total seed weight among them. Seed size was a major factor influencing total seed weight in these crosses. *Vaccinium ashei* female parents typically have large seeds, which tended to lead to a higher total seed weight than the much smaller seeds of *V. constablaei*; even though the seed number per berry was very high when *V. constablaei* was outcrossed, the total seed weight still was much less. F1 hybrids showed intermediate features in this regard. *Vaccinium simulatum* x *V. corymbosum* (highbush) crosses also produced small seeds and low total seed weight, comparable to crosses in which *V. constablaei* was the seed parent.

Total seed weight comparisons related to *V. constablaei* origin followed the same tendencies observed for the number of seeds per berry; higher values were obtained when clones from Shining Rock Wilderness Area were used as pollinizers for *V. ashei* clones than when clones from Roan Mt. were used (Table 2.14). Again, the differences were not statistically significant.

Number of Seedlings

Among all the fertility parameters used, the number of seedlings produced per pollinated flowers best measured the compatibility of a cross. The same conclusion was reached by Jelenkovic and Draper (1973) and Ballington and Galletta (1978). It was the only fertility parameter that revealed a ploidy barrier between the tetraploid *V. corymbosum* (highbush) as female parent and the hexaploids *V. ashei* and *V. constablaei* as pollen donors; these crosses produced far fewer seedlings than comparable crosses in which *V. ashei* was the female parent (Tables 2.7 and 2.10). Similar results were obtained by Lyrene (1988), who noticed that crosses between *V. corymbosum* as a tetraploid female parent with *V. ashei* generated a low number of seedlings, about 1/3 and 1/2 the number of seedlings per pollinated flower, as *V. corymbosum* and *V. ashei* intraspecific crosses, respectively. The data in Tables 2.7 and 2.10 show no significant differences between *V. ashei* intraspecific crosses compared to *V. ashei* x *V. constablaei* crosses, nor between *V. ashei* and *V. constablaei* as pollen parents when crossed with *V. corymbosum*. Despite the non-significant results, in crosses in which *V. corymbosum* and *V. ashei* were used as female parents, *V. constablaei* tended to induce the production of fewer seedlings than *V. ashei* when both were used as pollen donors. A similar tendency was observed with the number of seeds per berry and total seed weight as already discussed, suggesting that a weak crossing barrier may exist between *V. ashei* and *V. constablaei*. That more seedlings were produced when *V. constablaei* was crossed with *V. ashei* than when it was crossed with *V. corymbosum* probably reflects the difference between homoploid vs heteroploid crosses. The large number of seedlings obtained from

the *V. corymbosum* x *V. simulatum* cross indicates that *V. simulatum* was tetraploid. This was further evidenced by the very low number of seedlings produced by the *V. ashei* x *V. simulatum* cross.

No significant differences were observed between pollen sources when F1 (*V. ashei* x *V. constablaei*) hybrids were pollinated with *V. ashei* or F1 pollen in 1993, but the F1 pollen tended to produce more seedlings (Table 2.11). Both types of crosses significantly differed from selfed F1 lines, in which the total number of seedlings was greatly reduced. Selfed *V. ashei*, *V. constablaei* and F1 lines also produced far fewer seedlings compared to outcross pollination with *V. ashei* in 1994 (Table 2.12). These results agree only partially with El-Agamy et al. (1981), who mentioned that there was a trend, but no significant differences, toward higher germination in cross-pollinated than in self-pollinated *V. ashei* seed in their work. Payne et al. (1989) also noticed a reduction in the percentage of seed germination when two *V. ashei* cultivars were selfed compared to when they were intercrossed. *Vaccinium ashei* tended to be more severely affected by selfing than *V. constablaei* or the F1 hybrids (Table 2.12), although there were no statistically significant differences among selfed taxa regarding this characteristic. It appears that the number of seedlings per pollinated flower was the parameter that could best show the detrimental effects of inbreeding on the fertility of the selfed lines. Fertility was high in outcross pollinations, with no significant differences in the number of seedlings among *V. ashei*, F1 and *V. constablaei* lines when they were pollinated with *V. ashei*. F1 lines, however, tended to produce more seedlings than *V. ashei* in these

crosses, indicating a higher level of compatibility. The *V. simulatum* x *V. corymbosum* crosses produced, on average, very few seedlings.

Vaccinium constablaei from Shining Rock Wilderness Area tended to stimulate the production of more seedlings than clones from Roan Mt. when both were used to pollinate *V. ashei* clones, although the difference was not statistically significant (Table 2.14).

Altogether, the crossing experiments in the greenhouse show that *V. constablaei* and *V. simulatum* can be used in breeding *V. ashei* and *V. corymbosum*, respectively, for their crosses are viable and produce abundant, fertile, F1 progenies. Further improvement of selected F1 (*V. ashei* x *V. constablaei*) clones is possible, since they are highly fertile and have a high fruit set when crossed back to *V. ashei* or to a different F1 (*V. ashei* x *V. constablaei*) clone. Crosses between *V. ashei* x *V. simulatum* and *V. corymbosum* x *V. constablaei* are also feasible but they produce small progeny populations, presumably because of the different ploidy level of the parents. Selfing decreases fertility in *V. ashei*, *V. constablaei* and their F1 hybrids compared to outcross pollination. However, self-fertility averages higher in F1 (*V. ashei* x *V. constablaei*) hybrids compared to *V. ashei* clones, and this is a desirable characteristic of the F1 hybrids. The short fruit development period is another desirable characteristic present in the F1 hybrids, inherited from *V. constablaei*.

CHAPTER 3
SELF-FRUITFULNESS AND SELF-FERTILITY

Introduction

Self-fruitfulness is a desirable characteristic that needs to be improved in rabbiteye (*V. ashei*) cultivars, since most have low self-fruitfulness and give a low fruit set if not adequately cross-pollinated (Payne et al., 1989; Galletta and Ballington, 1996). Lyrene and Sherman (1984) stated that "if blueberry cultivation is to be successful in Florida, growers must be able to obtain high yields" (p. 322). Low yields in rabbiteye blueberries are frequent, though, and have been attributed to inadequate chilling (Lyrene and Crocker, 1983; Lyrene and Goldy, 1983; Lyrene and Sherman, 1985) and deficient outcross pollination with compatible cultivars (Garv ene, 1987; Payne et al., 1989).

Yields would probably be more consistent with self-fruitful cultivars even when cultivars for cross-pollination are provided (El-Agamy et al., 1979). Although most highbush clones have considerable self-fruitfulness, they benefit from cross-pollination by having increased fruit set and by producing larger fruits with early maturation and more seeds (Gupton, 1984; Lang and Danka, 1991).

Self-fruitfulness can be confused with self-fertility. They are distinct but usually correlated. Self-fruitfulness is defined here as the ability to set fruit without cross-

pollination. This definition includes fruit produced from self-pollination as well as parthenocarpic fruit. Self-fertility, on the other hand, is defined as the ability of a pollen grain to produce viable seed through selfing (Galletta and Ballington, 1996). In many blueberry cultivars, viable seed production is required for fruit development.

The terms "self-compatibility" or "self-incompatibility" have been used by researchers like Meader and Darrow (1944), Ballington and Galletta (1978), Garvey and Lyrene (1987) and Vander Kloet and Lyrene (1987) in order to refer to the reproductive fertility of blueberries after self-pollination. In recent reports, several researchers have advocated the idea that the use of the term "self-incompatibility" should be restricted to the existence of pre-fertilization barriers (Krebs and Hancock, 1988; Krebs and Hancock, 1990; Harrison et al., 1993; Harrison et al., 1994a; Harrison et al., 1994b). According to their observations, the term "self-incompatibility" would not apply to highbush and half-high blueberries, in which seed abortion seemed to occur after fertilization was completed in self-pollinated plants. El-Agamy et al. (1980) showed evidences of post-zygotic abortion also in rabbiteye (*V. ashei*) blueberries. The purpose of this chapter is to analyse and compare data regarding self-fruitfulness and self-fertility of *V. ashei*, *V. constablaei* and their F1 hybrids, in view of the fact that no information is available in the literature concerning self-fertility in *V. constablaei* and in *V. ashei* x *V. constablaei* F1 hybrids.

Materials and Methods

Most of the data discussed in this chapter came from experiments 5 and 6 carried out in the greenhouse in 1993 and 1994, respectively. In these experiments, selfing was accomplished in *V. ashei*, *V. constablaei* and their F1 hybrid lines according to the methodology described in chapter 2. Data are presented for each taxon, and parameters assessed include percentage fruit set, fruit development period, berry weight, number of seeds per berry, total seed weight and total number of seedlings.

A complementary set of *V. constablaei* selfs was carried out in the greenhouse in 1995, in order to test selfing in the clones that were not tested in experiment 6. These clones had been planted back in the field after completion of experiment 6 late in the summer of 1994. The four clones that were still alive on December 13, 1994, were again dug, potted and put in a cold room at about 7°C to satisfy their chill requirement. The plants were moved from the cold room on March 14, 1995 to the greenhouse where flowering took place. Eighty flowers were pollinated per plant. Fruits were harvested in May and June. Pollination and fruit picking dates were recorded as well as the number of berries harvested and their weight. The number of well-developed seeds was counted for the first 10 berries from each cross. The total seed weight was also recorded. A sample of 0.30g of seed per cross was treated with Captan, then planted in small pots filled with peat in the greenhouse on November 22. A mist irrigation system was used to keep the seeds wet. The temperature was maintained above 3°C, but the greenhouse was otherwise unheated. Later, the germinated seedlings were counted. Late-germinating seeds were also considered, until about two months after the first germinating seedlings

were counted and removed. The parameters used to evaluate this set of *V. constablaei* selfed clones were the same as mentioned above.

Results and Discussion

Selfing, in general, was detrimental to the fertility of *V. ashei*, *V. constablaei* and their F1 hybrids, while fruitfulness was not affected in some clones. There was, however, great variation among clones in this regard. A number of clones failed completely to set fruit. This included 58.3% of the *V. constablaei* clones tested, which was much higher than the 19.0% and 20.0% failure observed for F1 and *V. ashei* clones, respectively (Table 3.1). Two out of the 5 selfed *V. constablaei* clones that produced fruits were fairly self-fruitful, with about 47.0% fruit set on the average (Table 3.2), which was very close to the 55.5% fruit set obtained on *V. constablaei* after pollination with *V. ashei*. In the same way, seediness and the total number of seedlings produced were high after selfing three of the *V. constablaei* clones, but, in general, these characteristics were reduced when *V. constablaei* was selfed. The total number of seedlings was by far the most affected, and it was considered the most reliable characteristic to show the negative

Table 3.1 - Number and percentage of selfed *V. ashei*, *V. constablaei* and their F1 hybrid clones that completely failed to set fruit in the greenhouse.

Selfed lines	Total number of selfs	Selfs that failed (#)[z]	(%)
V. ashei	15	3	20.0
F1 hybrids	21	4	19.0
V. constablaei	12	7	58.3

[z] Selfs with zero fruit production.

Table 3.2 - Fertility measurements[Z] in *V. constablaei* clones in 1994 and 1995 after self-pollination compared to the mean fertility when eight *V. constablaei* clones of the same origin were pollinated with *V. ashei* in the greenhouse in 1994.[Y]

	Selfed *V. constablaei* clones[X]					*V. constablaei*
	1994		1995			x
Trait	93-333	93-338	93-332	93-42	93-43	*V. ashei*
Fruit set (%)	48.0	2.0	33.8	46.2	32.5	55.5
Fruit development period(d)	38.0	60.0	41.0	35.0	40.0	42.9
Berry weight (g)	0.24	0.17	0.36	0.59	0.61	0.42
Seeds/berry (#)	25.4	1.0	11.5	31.7	33.0	40.4
Seed wt/50 flowers (mg)	200	0.6	53	315	247	375
Seedlings/50 flowers (#)	499	0	55	485	469	852

[Z] Based on 50 pollinated flowers per clone in 1994 and 80 pollinated flowers per clone in 1995.
[Y] Six *V. constablaei* clones in 1994 and one in 1995 produced no fruit after self-pollination and were not included in this table.
[X] These *V. constablaei* clones are the same ones used in experiment #6 in the greenhouse from source (126), which originated from an open-pollinated seed composite of 100 clones from Shining Rock Wilderness Area.

effects of self-pollination. Berry weight and fruit development period are strongly influenced by berry seediness (Morrow, 1943; Harrison et al., 1993). Low number of seeds per berry tends to decrease berry weight and extend the fruit development period. These effects were evident in the *V. constablaei* clones that set fruit, but because almost all of them that produced fruit also produced reasonable amounts of seeds, they did not show large deviations for both traits compared to outcross pollination. Overall, *V. constablaei* showed a pattern similar to *V. angustifolium*, another wild blueberry species, in which 12 out of 21 lines were completely self-sterile and 9 had varying degrees of self-fertility according to a study by Aalders and Hall (1961). Nevertheless, *V. constablaei* clones produced far more seeds per berry when selfed than did *V. angustifolium* (lowbush) clones.

Most *V. ashei* clones showed severe reductions in fruit set, berry weight, seed number per berry, and seedling number per pollinated flower due to selfing. Two clones, however, stood out for being highly self-fruitful and for showing a slightly higher level of self-fertility compared to the others (Table 3.3). Some *V. ashei* clones were prone to produce parthenocarpic fruit, and parthenocarpy could explain the high fruit set in instances where the number of seeds per berry was low. Self-fruitfulness varied from 0% to 94%. Such variation has been reported before among selfed *V. ashei* clones. Meader and Darrow (1944) mentioned that out of ten *V. ashei* selfed clones, two produced no fruit, seven had fruit set ranging from 2.3% to 32.9% and one was quite self-fruitful, with a fruit set of 72.2%. Seed set was very low in all selfed *V. ashei* clones compared to outcross intraspecific pollination. Very few seedlings were obtained after selfing. The

Table 3.3 - Fertility measurements[z] in *V. ashei* clones after self-pollination compared to the mean fertility of the same *V. ashei* clones cross-pollinated with other *V. ashei* clones in the greenhouse in 1994.[y]

Fertility feature	Selfed *V. ashei* clones												*V. ashei* x
	92-285	92-265	92-251	85-101	92-164	92-255	92-273	92-282	92-279	92-258	92-252	92-254	*V. ashei*
Fruit set (%)	2	88	50	92	78	94	12	64	40	28	20	12	82.4
Fruit development period (d)	104	67	69	74	61	70	74	69	77	79	79	91	63.3
Berry weight (g)	0.91	1.30	1.39	1.80	1.49	1.26	1.73	1.35	1.33	0.87	0.79	0.97	1.93
Seeds/berry (#)	2	0.2	1.8	6.3	1.5	8.3	1.7	2.0	0.9	2.5	0.6	1.7	29.6
Seed wt/50 flowers (mg)	2.0	3.9	16.2	129.6	30.8	173.9	10.0	22.6	6.4	18.9	3.7	7.5	782
Seedlings/50 flowers (#)	2	6	19	166	33	231	8	34	7	24	4	8	895

[z] Based on 50 pollinated flowers per clone.

[y] Three *V. ashei* clones produced no fruit after self-pollination and were not included in this table.

fruit development period was extended in most clones, and berry weight was significantly reduced, both of which may have been due to low seed number per berry.

Selfed F1 (*V. ashei* x *V. constablaei*) hybrid clones also showed reductions in fruit set, berry weight, seed number per berry and seedling number per pollinated flower (Tables 3.4 and 3.5). Nevertheless, three of these clones had a high fruit set, 80% or above, which was remarkable in view of the fact that F1 clones did not form parthenocarpic fruits like some rabbiteye clones. High fruit set, however, did not guarantee high seed set and high seedling production, and a large variation could be found among F1 clones in this regard. One F1 clone had an average of 22.5 seeds per berry, which was considered high for a selfed clone, especially compared to *V. ashei*. In fact, one third of all F1 selfed clones produced more than 10 seeds per berry on average, and no self-pollinated *V. ashei* clone approached this level. The number of seedlings obtained by selfing the most self-fertile F1 clones was much greater than the number obtained by selfing *V. ashei* but much fewer than the number obtained by pollinating F1 clones with *V. ashei* pollen. The fruit development period and berry weight responses varied among clones. It was observed that some F1 clones with low seed per berry counts after selfing showed a decrease in berry weight and an increase in fruit development period compared to crossing. A significant negative correlation of -0.54 between seed number after selfing and decrease in berry weight, and a non-significant negative correlation of -0.46 between seed number after selfing and increase in fruit development period were obtained for the F1 hybrids ($P \leq 0.05$). Non-significant negative correlations of -0.32 and -0.25 were found for the same traits, respectively, for

Table 3.4 - Fertility measurements[Z] in *V. ashei* x *V. constablaei* F1 hybrids after self-pollination compared to the mean fertility of similar F1 hybrids pollinated with pollen from *V. ashei* in the greenhouse in 1993.[Y]

	Selfed *V. ashei* x *V. constablaei* F1 clones							F1 x
Fertility feature	93-95	93-99	93-101	93-109	93-110	93-112	93-117	*V. ashei*
Fruit set (%)	80	53	14	2	7	11	23	51.5
Fruit development period (d)	52	52	49	60	60	68	55	49
Berry weight (g)	0.89	0.40	0.57	0.34	0.59	0.49	0.88	1.05
Seeds/berry (#)	12.4	5.7	4.0	3.0	7.0	3.9	11.5	28.3
Seed wt/100 flowers (mg)	1200	290	29	3	24	21	109	760
Seedlings/100 flowers (#)	784	110	30	4	37	32	130	1039

[Z] Based on 100 pollinated flowers per clone.

[Y] One F1 hybrid produced no fruit after self-pollination and was not included in this table.

Table 3.5 - Fertility measurements[Z] in *V. ashei* x *V. constablaei* F1 hybrids after self pollination compared to the mean fertility of similar F1 hybrids pollinated with pollen from *V. ashei* in the greenhouse in 1994.[Y]

	Selfed *V. ashei* x *V. constablaei* F1 clones										F1 x
Fertility feature	94-47	94-58	94-59	94-48	94-61	94-57	94-56	94-49	94-45	94-55	*V. ashei*
Fruit set (%)	60	56	74	64	58	94	98	46	12	34	71.7
Fruit development period (d)	55	41	48	59	41	61	61	54	55	45	49
Berry weight (g)	0.55	0.74	0.99	0.59	0.97	0.82	0.83	0.65	0.21	0.81	0.82
Seeds/berry (#)	13.9	11.6	12.3	3.2	22.5	13.1	5.2	8.5	5.8	9.5	33.4
Seed wt/50 flowers (mg)	322	204	318	16	383	624	424	138	15	78	595
Seedlings/50 flowers (#)	332	219	473	27	483	366	137	147	31	129	1022

[Z] Based on 50 pollinated flowers per clone.

[Y] Three other F1 hybrids produced no fruit after self pollination and were not included in this table.

V. ashei selfed clones ($P \leq 0.05$). These correlations indicate that the clones that produced the most seeds per berry after selfing had the smallest decrease in berry weight and the smallest increase in fruit development period due to selfing.

The large variation found for self-fruitfulness and self-fertility within *V. ashei*, *V. constablaei* and their hybrid F1 lines indicates that selection for these characteristics is possible. The initial choice of parents is critical for such a breeding program according to Galletta (1970). It was observed by Galletta (1970) and Lyrene (1983b) that progenies from selfed lines had some vigorous seedlings, although most of the seedlings were weak. Our studies show that *V. constablaei* may be a good source of self fertility and was the principal source of self fertility in the *V. ashei* x *V. constablaei* F1 hybrids.

CHAPTER 4
OPEN-POLLINATED FERTILITY

Introduction

Fertility in open-pollinated blueberry field plantings varies depending on the female fertility of the clone being observed, the availability of viable, compatible pollen, and on whether cross-pollination predominates over self-pollination or vice-versa. Selfing tends to reduce fertility when compared with cross-pollination in highbush and rabbiteye clones (El-Agamy et al., 1981; Hellman and Moore, 1983; Krebs and Hancock, 1988; Harrison et al., 1993). Cross-pollination was recognized as a requirement for good blueberry production as early as 1921, when Coville, cited by Eck and Childers (1966), mentioned that berries were smaller and later in ripening when a blueberry plant was self-pollinated in contrast to when it was cross pollinated. Since then, interplanting two or more cultivars in *V. ashei* (rabbiteye) and *V. corymbosum* (highbush) field plantings has been recommended in order to enhance fruit production (Eck and Childers, 1966; Lyrene and Crocker, 1991).

Berry weight and development period are the two fruit characteristics most influenced by berry seediness (Morrow, 1943; Harrison et al., 1993) which, in turn, is a direct result of pollination effectiveness (Lyrene and Crocker, 1991) and can be an indication of cross-compatibility among cultivars (Darnell and Lyrene, 1989). In order to prevent problems with cross-pollination in open-pollinated field plantings, Lyrene and

Crocker (1991) suggested that blueberry growers should monitor berry seediness from each cultivar each year. This practice was also recommended by Darrow (1958), who stated that "seed numbers are useful in interpreting differences in fruit set and development in different localities and under different conditions" (p. 212). The principal reason for making the observations reported in this chapter was to determine whether or not the interspecific *V. corymbosum* x *V. simulatum* F1 hybrids had reduced female fertility compared to the *V. corymbosum* x *V. corymbosum* seedlings. High fertility for the hybrids would confirm that the *V. simulatum* parents were tetraploid and that *V. simulatum* is genetically close enough to *V. corymbosum* to avoid sterility problems when *V. simulatum* is used in breeding highbush cultivars.

Materials and Methods

Open-pollinated fertility was measured for 1.5-year old seedlings from five *V. corymbosum* x *V. corymbosum* and five *V. corymbosum* x *V. simulatum* crosses in a high-density field nursery at the Horticultural Unit of the University of Florida in Gainesville, FL, in 1994. These crosses are described in Table 4.1. Twenty F1 seedlings were randomly selected per cross when the fruit began to ripen, and a variable number of berries were randomly picked per plant, depending on fruit availability. The principal fertility parameter assessed was seed weight per 40 berries, based on seed extracted from 2 berries picked at random from each of 20 plants per cross. The weight of 100 well-developed seeds, picked from the seed extracted from the 40 berries was also measured to assist in the interpretation of the seed weight/berry data. Differences in fertility between

the two types of seedlings were tested by the analysis of variance according to a completely randomized design.

Table 4.1 - Description of the *V. corymbosum* x *V. corymbosum* and *V. corymbosum* x *V. simulatum* F1 progenies planted in a high-density nursery in Gainesville in 1993.

Progeny	Seed parent	Pollen parent
	(*V. corymbosum*)	(*V. corymbosum*)
1	91-16	87-217
2	82-217	81-83
3	92-79	91-156
4	92-97	6-19
5	92-81	90-171
	(*V. corymbosum*)	(*V. simulatum*)[Y]
6	HB cvs[Z]	92-286
7	Marimba	92-156 + 92-152 B
8	90-174	92-152 C
9	90-173	92-156
10	90-150	92-157

[Z] Composite of 6 crosses.
[Y] All originated from Grandfather Mt., North Carolina.

Results and Discussion

Blueberries normally have a 100 or more ovules per pistil, which are potentially capable of becoming seeds (Vorsa and Ballington, 1991; Darnell et al., 1992). If there are plenty of bees and an abundance of compatible pollen, seed number per berry can be a good measure of female fertility. Highly fertile plants of *V. ashei* (rabbiteye) and *V. corymbosum* (highbush) clones usually produce 15 to 30 well-developed seeds per berry (Moore et al., 1972; Kushima and Austin, 1979). On the other hand, low fertility plants such as *V. darrowi* x *V. arboreum* F1 hybrids (Lyrene, 1991), triploids (Dweikat and Lyrene, 1988) and pentaploids (Meader and Darrow, 1944; Vorsa et al., 1987) produce

few seeds. The number of well-developed seeds in these low fertility plants ranges from 0 to 2 seeds per berry.

F1 hybrids between *V. corymbosum* x *V. corymbosum* and *V. corymbosum* x *V. simulatum* appeared to have high fertility after open-pollination in Gainesville, FL (Table 4.2). The fact that the seed weight/40 berries was high and that no significant difference was found between the two types of hybrids indicates that *V. corymbosum* x *V. simulatum* F1 clones are fully female fertile. There were, however, significant differences in the weight of 100 seeds, which probably are primarily due to inherent differences in seed size between *V. corymbosum* and *V. simulatum* rather than differences in fertility levels. The average number of well-developed seeds per berry for each type of cross, estimated from the data in Table 4.2, was 35.4 and 39.1 for *V. corymbosum* x *V. corymbosum* and *V. corymbosum* x *V. simulatum* F1 hybrids, respectively. These values are quite high,

Table 4.2 - Comparison of F1's between *V. corymbosum* (highbush) x *V. corymbosum* and *V. corymbosum* x *V. simulatum* regarding open-pollinated fertility in the field in 1994.

	F1 clones	
	HB x HB	HB x *V. simulatum*
Number of crosses	5	5
Seed wt/40 berries (g)[z]	0.77[y]	0.70
Weight of 100 seeds (mg)	54.38 a	44.80 b

[z] Based on 2 random berries from each of 20 plants per cross.
[y] Means within a row followed by different letters are significantly different at $P \leq 0.05$ according to the analysis of variance.

demonstrating that both types of hybrids have high fertility. The high fertility of the *V. corymbosum* x *V. simulatum* F1 hybrid seedlings is an indication that the *V. simulatum*

clones from Grandfather Mt. are tetraploid, because they combined very well with the tetraploid *V. corymbosum* clones used as female parents in these crosses.

Observations regarding seed content of the fruit indicated that *V. corymbosum* x *V. simulatum* F1 hybrids were as fertile as *V. corymbosum* x *V. corymbosum* F1 hybrids when open-pollinated. The high fertility of the *V. corymbosum* x *V. simulatum* F1 hybrids further suggested that *V. simulatum* is tetraploid and that it can be used to breed *V. corymbosum* (highbush) cultivars, since fertility of the hybrids would not be a limitation.

CHAPTER 5
FRUIT QUALITY

Introduction

High fruit quality has always been a major goal in the development of blueberry cultivars. Galletta and Ballington (1996) included fruit quality traits in a list of the most important characters to be considered when breeding blueberries. Selection criteria for fruit quality used by the University of Florida breeding program for *V. corymbosum* (highbush) and *V. ashei* (rabbiteye) cultivars include large fruit with light blue color, small and dry picking scar, good firmness, no stems attached to the fruits after harvest, and good flavor.

Rabbiteye and highbush blueberries have differences in fruit quality. Although there is variability among populations and cultivars, rabbiteye fruit tend to have a smaller picking scar, larger seeds, higher percentage of soluble solids, better firmness and better color. The flesh of rabbiteye blueberry tends to have higher grittiness due to the large seeds and a moderately high number of sclereids (stone cells). Highbush fruit have larger size, higher acidity, smaller seeds, superior flavor and more tender flesh and skin (Ballington et al., 1984; Makus and Morris, 1987; Makus and Morris, 1993; Galletta and Ballington, 1996). Both *V. ashei* and *V. corymbosum* have some desirable fruit characteristics, and these species appear to complement each other in terms of fruit quality. The transfer of characteristics from *V. ashei* to *V. corymbosum* or vice-

versa has been difficult, however, since direct hybridization gives pentaploids in the F1 generation. These have reduced fertility, and when backcrossed to either of the parents, they tend to produce aneuploid plants (Vorsa et al., 1987; Vorsa, 1988; Laverty and Vorsa, 1991).

Crosses with other species, like *V. constablaei* and *V. simulatum*, might contribute to improved berry quality in rabbiteye and highbush blueberries. *Vaccinium ashei* x *V. constablaei* and *V. corymbosum* x *V. simulatum* crosses normally produce abundant F1 progeny, which are highly fertile. Fruits of *V. constablaei* were described by Brightwell et al. (1949) as having "a light blue color, tender skin and flesh, a very good flavor, small seeds and large size for a wild blueberry" (p. 239). They also have a low incidence of sclereids (Yarbrough and Morrow, 1947). *Vacccinium simulatum* fruit was described by Ballington (1990) as having "a pleasant subacid to acid flavor, and is quite variable for traits such as color and size" (p. 59).

Observations made by Brightwell et al. (1949) and Ballington et al. (1986) on the fruit quality of *V. ashei* x *V. constablaei* F1 hybrids revealed that, on the average, they were outstanding for picking scar and firmness, but not for fruit color. In all fruit quality traits, however, some seedlings were rated above average due to segregation. In general, these F1 hybrids produced smaller fruit than cultivated varieties of highbush and rabbiteye blueberries, but most hybrid progenies produced fruit with acceptable size for mechanical harvesting (Brightwell et al., 1949; Ballington et al., 1986). A wide variation in the number of sclereids was found by Yarbrough and Morrow (1947) in seedlings of *V. ashei* x *V. constablaei*. Therefore, selection of clones that produce fruit with no sclereids

would be possible, and this characteristic could be combined with other good fruit characteristics from *V. ashei* and *V. constablaei* in their F1 hybrids (Yarbrough and Morrow, 1947). This chapter compares and provides information about the quality of the fruit produced by *V. ashei*, *V. corymbosum*, *V. constablaei* and *V. simulatum* clones and some of their F1 hybrids in Gainesville, FL.

Materials and Methods

Berry color, firmness and picking scar were assessed in the fruit produced by the female parents in the crosses made in the greenhouse in 1993 and 1994. The crosses were described in chapter 2 (experiments 3, 4, 5 and 6). Altogether, these evaluations involved 25 *V. ashei*, 10 *V. constablaei*, 42 F1 hybrid (*V. ashei* x *V. constablaei*), 4 *V. corymbosum* and 3 *V. simulatum* clones. In addition, 5 to 30 seedlings from each of 10 *V. ashei* x *V. ashei* and 10 *V. ashei* x *V. constablaei* F1 progenies were assessed for fruit quality traits (color, firmness, picking scar and size) in an open pollinated high-density nursery in 1993. The progenies that were evaluated are described in Table 5.1. The high-density nursery was planted at the Horticultural Unit of the University of Florida in Gainesville, FL, on May 14, 1992.

Fruit quality was also evaluated in five *V. corymbosum* x *V. corymbosum* and five *V. corymbosum* x *V. simulatum* F1 progenies in 1994. Characters evaluated were berry color, firmness, picking scar, size, and presence of stems attached to the fruits after harvest. Twenty seedlings with ripe fruit were randomly selected per progeny. These seedlings were growing in a high-density nursery at the Horticultural Unit, planted on

Table 5.1 - Description of the seedling progenies and respective number of plants assessed for fruit quality traits in a high-density nursery in 1993 and 1994.

Cross	Seed parent	Pollen parent	Number of plants[z]
	(*V. ashei*)	(*V. ashei*)	
1	85-97	Baldwin	10 (8)
2	89-176	Powderblue	10 (7)
3	91-54	Premier	10 (6)
4	91-63	Brightwell	10 (8)
5	91-60	Brightwell	10 (8)
6	89-317	85-97	10 (5)
7	89-310	Premier	10 (9)
8	85-97	84-54 (wild)	10 (6)
9	91-49	W78-69	10 (9)
10	88-151	91-2	10 (8)
	(*V. ashei*)	(*V. constablaei*)	
11	89-191	NC 86-36-4	28 (27)
12	91-5	Corvallis # 8+10	10 (9)
13	85-97	Corvallis # 8	30 (21)
14	89-310	Corvallis # 8	10 (6)
15	85-97	Corvallis # 5	10 (9)
16	89-178	Corvallis # 8	10 (8)
17	91-2	Corvallis # 11	30 (30)
18	85-109	Corvallis # 8	10 (9)
19	89-178	Corvallis # 4	10 (9)
20	90-20	NC 86-36-4	10 (7)

[z] The number of plants outside parenthesis represent the ones marked in January of 1993 while still dormant; plants selected were the ones with most vigorous canes and most flower buds; the plants effectively assessed for fruit quality traits were the ones among those marked that flowered and produced fruit after a strong freeze on March 14 (number inside parenthesis).

May 27, 1993, and fruits were produced in an open pollinated condition. These progenies are described in Table 5.2.

Fruits were evaluated for quality shortly after they were collected. Color, firmness and picking scar were rated on a 9 point-scale. The score 1 was assigned to the least desirable feature of each trait, namely, a shiny black color, a very soft fruit, or a large, deep and wet picking scar with the surrounding skin torn in all examined fruits. A

Table 5.2. - Description of the *V. corymbosum* x *V. corymbosum* and *V. corymbosum* x *V. simulatum* F1 progenies assessed for fruit quality traits in a high-density nursery in 1994.

Progeny	Seed parent	Pollen parent
	(*V. corymbosum*)	(*V. corymbosum*)
1	91-16	87-217
2	82-217	81-83
3	92-79	91-156
4	92-97	6-19
5	92-81	90-171
	(*V. corymbosum*)	(*V. simulatum*)[Y]
6	HB cvs[Z]	92-286
7	Marimba	92-156 + 92-152 B
8	90-174	92-152 C
9	90-173	92-156
10	90-150	92-157

[Z] Composite of 6 crosses.
[Y] All originated from Grandfather Mt., North Carolina.

score of 5 corresponded to medium quality and the maximum score 9 matched the best quality that could be achieved for each trait. A score of 9 would correspond to a light-blue color with very adherent wax, very firm fruits or a small, shallow and dry picking scar in all examined fruits. A similar scale to assess fruit color, firmness and picking scar had been previously used by Ballington et al. (1986), Brightwell et. al. (1949), Draper et al., (1982), Edwards et al., (1974) and Finn and Luby (1992). Fruit size was assessed by weighing five berries picked at random per seedling. Fruits with attached stems were counted per seedling after harvest in such a way that the percentage of seedlings and berries showing this characteristic could be calculated. Differences between types of crosses for mean berry weight, and percentage of seedlings and berries with attached stems were tested by the analysis of variance and, if significance was achieved, means were compared by the Tukey-test.

Results and Discussion

Fruit Color

Light-blue color is desirable in blueberry fruits, especially if the fruit is intended for the fresh market. The intensity of the blue color is determined by wax on the skin of the fruit. Light-blue berries have a thick layer of wax of a particular type, as shown by the electron micrographs of Albrigo et al. (1980). The surface wax may retard moisture loss and shriveling of the fruits after harvest. Black fruits, on the other hand, lack the type of wax associated with blue fruit color. Although it is rarely mentioned, selection of clones with light-blue color should take into account the adherence of the layer of wax on the surface of the fruit so that the wax is not easily removed during picking and handling.

Among the taxa surveyed in the greenhouse in 1993 and 1994, *V. ashei*, *V. corymbosum*, *V. constablaei* and *V. simulatum* had fruit with blue color. Most *V. ashei* x *V. constablaei* F1 clones, however, tended to produce black fruit (Table 5.3). These results show that the pattern of fruit color differs between F1 progenies from *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* crosses. Confirmation of this difference was obtained

Table 5.3 - Distribution of fruit color among *V. ashei*, *V. constablaei* and their F1 hybrids, *V. corymbosum* (highbush) and *V. simulatum* clones used as seed parents in experiments 3, 4, 5 and 6 in the greenhouse in 1993 and 1994.

	Number of plants with color score[2]									
Seed parent	1	2	3	4	5	6	7	8	9	Total
V. ashei	0	0	0	0	3	5	13	4	0	25
V. constablaei	0	0	0	1	2	3	3	1	0	10
F1 (ash x const)	0	12	12	12	1	2	3	0	0	42
V. corymbosum	0	0	0	1	0	1	1	1	0	4
V. simulatum	0	0	0	1	0	2	0	0	0	3

[2] Fruit color was rated on a 9 point-scale, 1 meaning a poor color (shiny black), 5 is a medium color (toward blue but wax comes off easily) and 9 the desirable one (blue with very adherent wax).

when segregating seedling progenies of these two types were compared in a high-density nursery in 1993. While 89% of the *V. ashei* x *V. ashei* seedlings produced fruit rated above medium for blue color, only 26% of the *V. ashei* x *V. constablaei* F1 seedlings received that score. Fruit with black color was produced by most *V. ashei* x *V. constablaei* F1 seedlings in the 1993 field nursery evaluation (Table 5.4).

Table 5.4 - Distribution of fruit color among F1 plants from *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* crosses in a high-density nursery in 1993.

	Number of plants with color score[z]									
F1 parent	1	2	3	4	5	6	7	8	9	Total
V. ashei x *V. ashei*	0	3	0	4	1	14	32	20	0	74
V. ashei x *V. constablaei*	0	27	21	28	24	20	14	1	0	135

[z] Fruit color was rated on a 9 point-scale, 1 meaning a poor color (shiny black), 5 is a medium color (toward blue but wax comes off easily) and 9 the desirable one (blue with very adherent wax).

Seedlings that produced black fruit also predominated within segregating *V. corymbosum* x *V. simulatum* F1 progenies evaluated in another high-density nursery in 1994. Although *V. corymbosum* x *V. corymbosum* F1 seedling progenies segregated for fruit color, 63.0% of their seedlings produced fruit with blue color rated above medium (Table 5.5).

Table 5.5 - Distribution of fruit color among F1 plants from *V. corymbosum* (highbush) x *V. corymbosum* and *V. corymbosum* x *V. simulatum* crosses in a high density nursery in 1994.

	Number of plants with color score[z]									
F1 parent	1	2	3	4	5	6	7	8	9	Total
HB x HB	0	5	10	12	10	27	30	6	0	100
HB x *V. simulatum*	9	35	21	24	8	3	0	0	0	100

[z] Fruit color was rated on a 9 point-scale, 1 meaning a poor color (shiny black), 5 is a medium color (toward blue but wax comes off easily) and 9 the desirable one (blue with very adherent wax).

The fact that a large number of seedlings from *V. ashei* and *V. corymbosum* intraspecific crosses had berries with light-blue color could be in part explained because the parents used had been previously selected for this feature in the Florida blueberry breeding program. The segregation pattern for fruit color in these intraspecific crosses is possibly due to additive gene action, similar to the pattern observed by Finn and Luby (1992) in progenies from highbush x highbush or half-high x half-high blueberry crosses. Finn and Luby observed that the fruit color scores from those progenies tended to concentrate between the two parental color scores, with a few clones segregating outside of these limits. They also reported that the progeny from two light-blue parents tended to segregate around the higher parental color rating, and that, when one parent is dark-blue and the other light-blue, the progeny ranged between and around the two parental scores.

Epistatic gene action that changed the wax on the fruit surface could account for the predominance of seedlings with black fruit from *V. ashei* x *V. constablaei* and *V. corymbosum* x *V. simulatum* crosses, since the parental clones used in these interspecific crosses were all blue-fruited. This epistatic effect had been previously reported by Ballington et al. (1986) among *V. ashei* x *V. constablaei* F1 seedlings from a cross where both parents had light-blue fruit. A poor color (toward black) was also observed in most *V. ashei* x *V. constablaei* seedlings obtained by Brightwell et al. (1949), but in that case it could be attributed to the use of a black-fruited rabbiteye as one parent. Ballington (1980) mentioned that pentaploid hybrids between *V. corymbosum* and *V. ashei* generally produce black fruit. Ballington also observed that blue-fruited *V. ashei* x *V. constablaei* F1 hybrid clones crossed with blue-fruited highbush clones produce some black and some

blue-fruited pentaploids (Lyrene, 1993). Finn and Luby (1992) reported that lowbush parents (*V. angustifolium*) having light-blue fruit produced segregating progeny with mostly black fruit whether the other parent was a highbush or a half-high clone and whether its fruit was blue or black. These observations, together with our results, suggest that black-fruited seedlings can normally be expected from blueberry interspecific crosses. Draper (1977), however, mentioned that *V. darrowi* x highbush tetraploid hybrids frequently have blue fruit. The inheritance of fruit color in blueberries does not seem to follow a single pattern and is not yet well understood.

Selection for fruit color appears possible in *V. ashei* x *V. constablaei* and *V. corymbosum* x *V. simulatum* progenies, since they showed segregation for this trait in the F1 generation. Darrow et al. (1952) reported that a few seedlings from two *V. ashei* x *V. constablaei* progenies had fruits with a fine blue color, and Ballington et al. (1986) observed a few seedlings with superior color from another *V. ashei* x *V. constablaei* F1 progeny.

Picking Scar

Picking scar is the opening that remains in the fruit surface when it is detached from the pedicel during harvest. Small, dry and shallow picking scars are desirable. Large, wet scars with torn skin favor the entry of decay organisms. This type of scar may also favor rapid moisture loss from the fruit.

Vaccinium ashei, *V. constablaei*, *V. simulatum* and *V. ashei* x *V. constablaei* F1 hybrid clones used as seed parents in the greenhouse in 1993 and 1994 tended to show a desirable small and dry scar. Some variation was observed among *V. corymbosum*, *V.*

constablaei and *V. ashei* x *V. constablaei* F1 hybrid clones, with a few of them having a bad or medium scar (Table 5.6).

Table 5.6 - Picking scar evaluation of fruits of *V. ashei*, *V. constablaei* and their F1 hybrids, *V. corymbosum* (highbush) and *V. simulatum* clones used as seed parents in experiments 3, 4, 5 and 6 in the greenhouse in 1993 and 1994.

	Number of plants with picking scar score[z]									
Seed parent	1	2	3	4	5	6	7	8	9	Total
V. ashei	0	0	0	0	0	0	1	24	0	25
V. constablaei	1	0	1	0	0	0	5	3	0	10
F1 (ash x const)	0	0	2	1	2	2	5	27	3	42
V. corymbosum	0	0	0	1	0	2	0	1	0	4
V. simulatum	0	0	0	0	0	0	1	2	0	3

[z] Picking scar was rated on a 9 point-scale, 1 meaning a bad scar (tears the surrounding skin easily and is deep and humid), 5 is a medium situation and 9 the desirable one (small and dry in all fruits).

Small, dry picking scars were also observed in most seedlings within segregating F1 progenies from *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* crosses evaluated in a high-density nursery in 1993 (Table 5.7). Less segregation for this trait was observed, however, within *V. ashei* populations compared to *V. ashei* x *V. constablaei* F1 hybrid populations. A small, dry picking scar is typical for rabbiteye blueberries. In addition, the *V. ashei* parents used in the crosses came from the Florida blueberry breeding program, and had been under selection for small and dry picking scar for several

Table 5.7 - Picking scar evaluation on fruits of F1 plants from *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* crosses in a high-density nursery in 1993.

	Number of plants with picking scar score[z]									
F1 parent	1	2	3	4	5	6	7	8	9	Total
V. ashei x *V. ashei*	0	0	0	2	4	3	9	56	0	74
V. ashei x *V. constablaei*	1	14	4	10	11	19	39	37	0	135

[z] Picking scar was rated on a 9 point-scale, 1 meaning a bad scar (tears the surrounding skin easily and is deep and humid), 5 is a medium situation and 9 the desirable one (small and dry in all fruits).

generations. On the other hand, the *V. constablaei* clones used as male parents in the interspecific crosses with *V. ashei* had not undergone such selection, and this could have favored more segregation in the F1 hybrid population. Our results confirm those of Brightwell et al. (1949) and Ballington et al. (1986), who found a significant number of seedlings with small and dry scars within populations of *V. ashei* and their hybrids with *V. constablaei*. It seems that both *V. ashei* and *V. constablaei* can contribute favorable genes for this characteristic to their F1 hybrids as long as parents with small and dry scar are used. Selection of *V. ashei* x *V. constablaei* F1 clones with small and dry picking scar should be easy due to the predominance of clones with this characteristic.

Wide segregation was also observed in both *Vaccinium corymbosum* x *V. corymbosum* and *V. corymbosum* x *V. simulatum* F1 seedling populations assessed for picking scar in another high-density nursery in 1994. About 64% and 52%, respectively, of the seedlings of the two types were rated above medium for small and dry scars (Table 5.8). The highbush parents used in this study, like the rabbiteye parents, came from the Florida blueberry breeding program and had been under selection for small and dry picking scars for several generations. Highbush blueberries, however, typically have larger scars than rabbiteye, and this explains in part why a small and dry picking scar is not yet fixed in the highbush germplasm. The *V. simulatum* clones, used as pollen parents in the interspecific crosses with highbush blueberries, had not previously been selected for picking scar. Thus, it was surprising to find that the F1 population from the *V. corymbosum* x *V. simulatum* crosses, while highly variable for picking scar, was not

much worse than the F1 population from *V. corymbosum* x *V. corymbosum* crosses with respect to scar (Table 5.8).

Table 5.8 - Picking scar evaluation on fruits of F1 plants from *V. corymbosum* (highbush) x *V. corymbosum* and *V. corymbosum* x *V. simulatum* crosses in a high density nursery in 1994.

	Number of plants with picking scar score[z]									
F1 parent	1	2	3	4	5	6	7	8	9	Total
HB x HB	10	6	6	5	9	9	24	31	0	100
HB x *V. simulatum*	14	4	10	9	9	10	22	22	0	100

[z] Picking scar was rated on a 9 point-scale, 1 meaning a bad scar (tears the surrounding skin easily and is deep and humid), 5 is a medium situation and 9 the desirable one (small and dry in all fruits).

Fruit Firmness

Firmness is an essential quality in blueberries. High firmness permits the fruit to better withstand handling and shipping to the markets. The *V. ashei*, *V. corymbosum*, *V. simulatum* and *V. ashei* x *V. constablaei* F1 hybrid clones assessed in the greenhouse in 1993 and 1994 tended to have firm fruit. On the other hand, the *V. constablaei* fruits ranged from soft to medium in firmness (Table 5.9). Although it appeared that *V. simulatum* fruits were relatively firm, the fact that the clones used produced very small fruits probably contributed to their firmness. Larger fruits from *V. simulatum* clones would probably have been softer, like *V. constablaei* fruits.

Both *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 progenies assessed in a high-density nursery in 1993 showed a tendency to segregate toward high fruit firmness (Table 5.10). High firmness was probably inherited from the *V. ashei* parents, which typically have firm fruits. Besides, the *V. ashei* clones used as parents in the crosses had

been selected for higher firmness for several generations as part of the Florida blueberry breeding program, and this may have contributed to the lower variation found among the segregating *V. ashei* x *V. ashei* F1 progenies compared to *V. ashei* x *V. constablaei* progenies for this trait. The fact that *V. constablaei* fruits are less firm than *V. ashei* fruits could have been the reason for the higher variation found among the *V. ashei* x *V. constablaei* progenies.

Table 5.9 - Distribution of fruit firmness among *V. ashei*, *V. constablaei* and their F1 hybrids, *V. corymbosum* (highbush) and *V. simulatum* clones used as seed parents in experiments 3, 4, 5 and 6 in the greenhouse in 1993 and 1994.

	Number of plants with firmness score[z]									
Seed parent	1	2	3	4	5	6	7	8	9	Total
V. ashei	0	0	0	0	0	0	4	21	0	25
V. constablaei	0	1	2	1	4	2	0	0	0	10
F1 (ash x const)	0	0	1	0	0	0	6	35	0	42
V. corymbosum	0	0	0	0	0	1	0	3	0	4
V. simulatum	0	0	0	0	0	0	3	0	0	3

[z] Firmness was rated on a 9 point-scale, 1 corresponding to very soft, 5 is medium, and 9 a desirable very firm fruit.

Table 5.10 - Distribution of fruit firmness among F1 plants from *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* crosses in a high-density nursery in 1993.

	Number of plants with firmness score[z]									
F1 parent	1	2	3	4	5	6	7	8	9	Total
V. ashei x *V. ashei*	0	0	0	0	8	5	17	43	1	74
V. ashei x *V. constablaei*	0	1	1	4	20	17	42	50	0	135

[z] Firmness was rated on a 9 point-scale, 1 corresponding to very soft, 5 is medium, and 9 a desirable very firm fruit.

The great majority of the *V. corymbosum* x *V. corymbosum* and *V. corymbosum* x *V. simulatum* F1 clones evaluated in a high-density nursery in 1994 produced firm fruits (Table 5.11). This quality probably originated from the *V. corymbosum* parents used in

the crosses, which had been selected for high fruit firmness in the Florida blueberry breeding program. Some segregation was, however, observed within the progenies of both types of hybrids; a few clones produced soft fruits or fruits with medium firmness. *Vaccinium simulatum* may have contributed to the high firmness that predominated among the *V. corymbosum* x *V. simulatum* F1 hybrid clones.

Table 5.11 - Distribution of fruit firmness among F1 plants from *V. corymbosum* (highbush) x *V. corymbosum* and *V. corymbosum* x *V. simulatum* crosses in a high-density nursery in 1994.

	Number of plants with firmness score[z]									
F1 parent	1	2	3	4	5	6	7	8	9	Total
HB x HB	0	0	1	1	7	6	16	68	1	100
HB x *V. simulatum*	0	0	0	3	4	14	23	56	0	100

[z] Firmness was rated on a 9 point-scale, 1 corresponding to very soft, 5 is medium, and 9 a desirable very firm fruit.

Fruit size

Fruit size is important in the development of new blueberry cultivars. Large fruit is more appealing to growers and consumers and makes hand-harvesting much easier, especially when the fruit is intended for the fresh market. Mechanically harvested rabbiteye and highbush blueberries for the processed market do not necessarily need large fruit, but a minimum average berry weight of 1g is desirable, according to Galletta and Ballington (1996). Small berries tend to be preferred for processing markets; they are more suitable for making baked products than large berries (Lyrene, 1994c).

No significant difference was found for berry weight between *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 populations in an open-pollinated field in Gainesville, FL, in 1993 (Table 5.12). Berries from *V. ashei* x *V. constablaei* F1 plants were slightly

smaller, on average, than berries from *V. ashei* x *V. ashei* F1s. A few hybrids in almost every cross of the two types, however, produced fruit weighing 1g or more, showing that clones with acceptable berry size are available for selection. The berry size data obtained for *V. ashei* x *V. constablaei* F1 hybrids confirmed previous observations made by Ballington et al. (1986) in an F1 population of this type, which had an average berry weight of 0.93g.

Table 5.12 - Comparison of segregating F1 progenies between *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* regarding mean fruit weight in an open pollinated field condition in 1993.

	F1 clones	
	V. ashei x *V. ashei*	*V. ashei* x *V. constablaei*
Number of crosses	10	10
Mean berry weight (g)[Z]	1.15[Y]	0.90

[Z] Based of 5 random berries from each of a number of plants per cross, which varied from 5 to 30.
[Y] The analysis of variance shows no significant differences between the means at $P \leq 0.05$.

The mean berry weight observed for *V. ashei* x *V. ashei* F1 hybrids (1.15g) was lower than might have been expected, considering that cultivated rabbiteye varieties normally produce heavier fruits. This low berry weight observed in the *V. ashei* F1 populations can be explained by regression. That is, the parental clones had been selected in the blueberry breeding program from large seedling populations, with large berry size being an important selection criterion. When these parents were crossed, there was a tendency for the mean berry size of the progeny to be intermediate between the berry size of their parents and the mean berry size of the seedling population from which those

parents had been selected. Regression is commonly observed in the progeny when heterozygous parents that have been strongly selected for a quantitative trait are crossed.

Segregating F1 seedlings from *V. corymbosum* x *V. corymbosum* crosses differed significantly from *V. corymbosum* x *V. simulatum* F1 seedlings regarding mean berry weight after open pollination in the field in 1994 (Table 5.13). *Vaccinium corymbosum*

Table 5.13 - Comparison of segregating F1 progenies between *V. corymbosum* (highbush) x *V. corymbosum* and *V. corymbosum* x *V. simulatum* regarding mean berry weight and berries with attached stems in an open-pollinated field condition in 1994.

	HB x HB	HB x *V. simulatum*
Number of crosses	5	5
Mean berry weight (g)[Y]	1.54 a[Z]	0.91 b
Plants with stemmy berries (%)	21.00	22.00
Berries with attached stems (%)[X]	9.57	14.30

[Z] Means within a row followed by different letters are significantly different at $P \leq 0.05$ according to the analysis of variance.
[Y] Based on 5 random berries from each of 20 plants per cross.
[X] Based only on plants in which one or more berries had attached stems after harvested.

typically has large berries, even larger than rabbiteye blueberries, and it was expected that this characteristic would be expressed in the F1 generation of the intraspecific crosses. As expected, *V. corymbosum* x *V. simulatum* F1 hybrids had, on average, smaller berries. This berry size possibly reflected an interaction of the parental genes, and was presumed to be intermediate between the large fruited highbush parents and the smaller fruited wild *V. simulatum* parents.

Fruits with Attached Stems

Stems that remain attached to the fruit after harvest cause problems in the blueberry packing house. "Stemminess" occurs when the point of detachment of the fruit is not at the junction of the pedicel with the fruit but at the point where the pedicel attaches to the peduncle. If the berries are stemmy, extra labor is required to remove the stem before the fruit is eaten.

Vaccinium corymbosum x *V. corymbosum* and *V. corymbosum* x *V. simulatum* F1 clones did not differ significantly either in the percentage of berries with attached stems or in the percentage of clones bearing fruits with this characteristic (Table 5.13). Berries with stems that remain attached after harvest are commercially undesirable, but it appears that using *V. simulatum* as a parent did not increase the incidence of this problem. Fruit with attached stems did not occur in *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 progenies, and it appears that stemminess is not a widespread problem with these blueberry species.

Fruit characteristics that predominated among F1 seedlings from *V. ashei* x *V. constablaei* and *V. corymbosum* x *V. simulatum* in this study were black color, high firmness, small and dry picking scar, and fruit size intermediate between the two parents. Berries with attached stems were observed in some *V. corymbosum* x *V. simulatum* seedlings. Since both types of interspecific hybrids showed segregation for all fruit quality traits assessed, selection for desirable fruit characteristics is possible in the F1 generation.

CHAPTER 6
FLOWER MORPHOLOGY

Introduction

Researchers believe that fruit set in blueberries is related to the flower structure and attractiveness to pollinating insects. The blueberry flower is morphologically adapted to facilitate cross-pollination by bees. The distance between the stigma and the anther pores from which the pollen is discharged and the typical stigma shape with angled sides like an inverted funnel reduce within-flower self pollination. Although in the blueberry flower the anthers closely surround the pistil, in the absence of insects most of the pollen grains fall without landing on the stigma of the same flower (Eck and Childers, 1966).

Few studies have attempted to relate morphological features of the blueberry flower to fruit set percentage. Eck and Mainland (1971) measured various flower parts from 35 *V. corymbosum* (highbush) cultivars and observed that a high fruit set was correlated with a short distance between stigma and anther pore. High fruit set was also associated with short corollas that are wide at the middle and narrow at the base. Flowers with these characteristics possibly were more attractive to pollinating insects, and could easily be pollinated. According to Lyrene (1994a), long corolla tubes with narrow apertures are typical of rabbiteye blueberries and may reduce pollination success

rates by hindering honeybee entry into the flowers to reach the nectar and pollen. He also mentioned that it should be possible to improve the rabbiteye flower shape since there is much variation among clones for most flower structural features. *Vaccinium constablaei* could be used to breed rabbiteye cultivars with improved flower morphology, since *V. ashei* x *V. constablaei* F1 hybrids have floral features that appear to favor pollination by honeybees. Environmental factors can also change structural features of the blueberry flower but not enough to significantly affect fruit set (Lyrene, 1994b). In the present chapter, several flower morphological features of *V. ashei*, *V. constablaei*, their F1 hybrids, *V. corymbosum* and *V. simulatum* are described and compared to provide additional information on this subject.

Materials and Methods

Flower features were determined in 23 *V. ashei*, 12 *V. constablaei*, 13 F1 hybrids between them, 4 *V. corymbosum* and 1 *V. simulatum* clones that had been used as male and/or female parents in experiments 3, 4 and 5 carried out in 1992 and already described in chapter 2. The *V. constablaei* and *V. simulatum* flowers originated from branches cut from plants growing wild in the mountains of western North Carolina. The *V. ashei* and *V. corymbosum* were cultivars or advanced selections from the Florida breeding program. Ten fully opened flowers were assessed per clone. The following features were evaluated: corolla length, measured from the deepest indentation of the calyx to the apex of the corolla; corolla width, representing the widest diameter at the middle of the corolla tube; corolla aperture, which was the mean diameter of the opening at the apex of the

corolla tube; style length, which extended from the top of the ovary to the far end of the stigma (including stigma); anther-to-stigma distance, measured from the pore of the anthers up to the stigma; overall stigma position in relation to the corolla apex and color of the petals.

Statistical analyses were performed for all characteristics, except stigma position and color of the petals, based in a completely randomized design with variable replication. Significant differences among taxa were compared using Tukey's mean separation procedure.

Results and Discussion

Vaccinium ashei, *V. constablaei*, their F1 hybrids, *V. corymbosum* (highbush) and *V. simulatum* showed very distinct flower morphological features. *Vaccinium ashei* and *V. corymbosum* had larger flowers, as measured by the length of the corolla and style (Table 6.1). This large size may have been due, in part, to genetic changes caused by breeding cultivars with large fruit. Flowers of *V. ashei* and *V. corymbosum* (highbush) differed significantly from *V. constablaei* and F1 flowers, which were smaller. Highbush flowers stood out for their corolla width, which was significantly larger than the flowers of the other taxa. Despite their small size, *V. constablaei* flowers presented a large corolla aperture, similar to the aperture of highbush flowers and significantly larger than the aperture of *V. ashei* flowers. The anther pore, from where the pollen is released, was much closer to the stigma in *V. constablaei* than in the cultivated blueberries *V. ashei* and *V. corymbosum* (highbush).

Table 6.1 - Means of the clones sampled to estimate flower morphological features for *V. ashei*, *V. constablaei*, their F1 hybrid, *V. corymbosum* (highbush) and *V. simulatum*.

	Vaccinium taxa				
Flower feature	*V. ashei*	F1 (ash x const)	*V. constablaei*	Highbush	*V. simulatum*[Y]
Number of clones sampled	23	13	12	4	1
Corolla length (mm)	9.10 a[Z]	7.24 b	5.26 c	8.88 a	4.80
Corolla width (mm)	6.55 b	6.17 bc	5.21 c	7.91 a	6.08
Corolla aperture (mm)	2.74 b	3.32 ab	3.81 a	3.65 a	4.09
Style length (mm)	10.03 a	7.86 b	5.21 c	9.08 a	5.14
Anther-to-stigma distance (mm)	2.82 a	1.45 b	0.67 b	2.91 a	0.44

[Z] Means within a row followed by different letters are significantly different at $P \leq 0.05$ using the Tukey test.
[Y] Data from *V. simulatum* are not included in the statistical analyses because of insufficient replication but are given for comparison.

The data show that *V. constablaei* flowers have several desirable features like large aperture and short anther-to-stigma distance. Eck and Mainland (1971) and Lyrene (1994a) suggested that short and wide corollas, short distance between stigma and anther pore and large corolla-tube apertures could make the blueberry flower more attractive to pollinating insects, resulting in more efficient pollination and higher fruit set. The *V. ashei* flower lacked these features but it should be possible to modify this through crosses with *V. constablaei.* In the present study, mean values for all flower parameters measured in the F1 (*V. ashei* x *V. constablaei*) hybrids were intermediate between the two parents.

Vaccinium ashei and *V. constablaei* also differed in petal color (Table 6.2) and stigma position in relation to the corolla apex (Table 6.3). *Vaccinium ashei* clones tend to have white petals and stigmas exserted beyond the corolla apex. *Vaccinium constablaei*, on the other hand, had light-yellow flowers and stigmas even with the corolla apex. It is not known what effect these two flower characteristics have on insect attraction and pollination in blueberries. F1 hybrids had some variation in petal color and stigma position relative to the corolla apex as a result of segregation; therefore, selection in the F1 generation should be possible.

The small corolla tube apertures and stigmas exserted beyond the corolla apex indicated that *V. ashei* flowers were not designed to be penetrated by pollinating insects to effect pollination, as required by honeybees in order to reach the anthers (Cane et al., 1993). Instead, the morphological features of the *V. ashei* flowers are best adapted for pollination by sonicating bees, some of which are native to the *V. ashei* natural habitats

(Cane and Payne, 1990). Pollination carried out by these native bees is very effective in plant species with flowers that shed pollen through terminal pores in the anthers , like blueberries, and depends solely on the vibration caused by their wings when visiting the flowers (Corbet et al., 1988). The vibration causes the pollen to pour out of the anthers

Table 6.2 - Number of clones having various corolla colors for *V. ashei*, *V. constablaei*, their F1 hybrid, *V. corymbosum* (highbush) and *V. simulatum*.

Vaccinium taxon	Corolla color				Total number of clones
	White	Light-yellow	White w/ yellow veins	Red-yellow	
V. ashei	22	1	0	0	23
F1 (ash x const)	4	7	2	0	13
V. constablaei	1	11	0	0	12
V. corymbosum	2	1	0	1	4
V. simulatum	0	1	0	0	1

onto the head of the bees, and from there the pollen is transferred to the stigmas (Lyrene and Crocker, 1991). Sonicating bees are effective pollinators of *Vaccinium corymbosum* (highbush) blueberries, which also seem to be efficiently pollinated by honeybees (Goodman and Clayton-Green, 1988). Differences in honeybee attractiveness among highbush cultivars exist, however, which have been suggested to be related, in part, to differences in the intensity of nectar production (Marucci and Moulter, 1977; Jablonski et al., 1985). Honeybee efficiency in pollinating highbush flowers can be explained by the large corolla aperture and large corolla width of the cultivated highbush flower (Table 6.1). These two characteristics should facilitate honeybee entrance into the flowers to get

Table 6.3 - Stigma position in relation to the corolla apex of flowers of *V. ashei*, *V. constablaei*, their F1 hybrid, *V. corymbosum* (highbush) and *V. simulatum* clones.

Vaccinium taxon	Number of sampled clones with stigma position					Total number of clones
	Inside corolla tube	Inside or at the corolla apex	At the corolla apex	Exserted beyond or at the corolla apex	Exserted beyond corolla	
V. ashei	0	0	1	10	12	23
F1 (ash x const)	0	1	4	3	5	13
V. constablaei	0	0	12	0	0	12
V. corymbosum	0	1	1	2	0	4
V. simulatum	0	0	1	0	0	1

the pollen and nectar, consequently increasing pollination. Morphological features of *V. constablaei* flowers, like the large corolla apertures and stigmas at the same level of the corolla apex, could indicate that these flowers are adapted to pollinating insects other than sonicating bees, without discarding the fact that sonicating bees can be excellent pollinators for this species. Furthermore, it has been noticed that, although *V. constablaei* and *V. simulatum* are distinct species native to the mountains of North Carolina with very different growth habits, *V. constablaei* being a shrub less than 2m tall with hundreds of stems per mature clone and *V. simulatum* a short tree, growing up to 4m tall with few canes per clone, their flowers were much alike. Thus, it is further suggested that the flowers of both species evolved and are well-adapted to a similar pollination system in harmony with the pollinating insects natural to those mountains.

Flower morphological features of the F1 (*V. ashei* x *V. constablaei*) hybrids were intermediate between both parents. Compared to *V. ashei*, F1 (*V. ashei* x *V. constablaei*) hybrids had flower characteristics more favorable for honeybee pollination, such as larger aperture and smaller distance between stigma and anther pore.

CHAPTER 7
POLLEN FERTILITY AND UNREDUCED GAMETES

Introduction

Pollen stainability with aceto-carmine is frequently used to estimate pollen fertility. The fact that a pollen grain stains means that it has a cytoplasm. However, a pollen grain could stain well but still be unable to germinate and form a pollen tube. Thus, pollen stainability can give higher estimates of pollen fertility than direct tests of pollen germination, as verified by Rousi (1967). Nevertheless, the correlation between pollen fertility and pollen stainability is high enough to make pollen stainability a useful estimate of pollen fertility (Cockerham and Galletta, 1976).

Cockerham and Galletta (1976) surveyed pollen stainability in diploid, tetraploid, hexaploid and heteroploid blueberry species and hybrids. They found that pollen from tetraploids and hexaploids had a higher mean percentage of stainable pollen (85% and 82%, respectively) than diploids (70%), although pollen stainability among clones showed much variation. Heteroploids originated from crosses between *V. vacillans* (4x) x *V. ashei* (6x) and *V. corymbosum* (4x) x *V. ashei* (6x) had poor pollen stainability (less than 33%). Cockerham and Galletta (1976) suggested that the pollen of heteroploid hybrids has low stainability and, consequently, low fertility, due to irregularities in chromosome pairing during meiosis and segregation.

Pollen from diploid, tetraploid and hexaploid *Vaccinium* species has also been surveyed for the presence of unreduced gametes, primarily 2n, which are gametes with the sporophytic chromosome number (Ballington and Galletta, 1976; Cockerham and Galletta, 1976; Megalos and Ballington, 1987; Ortiz et al., 1992a; Ortiz et al., 1992b). Lyrene and Ballington (1986) mentioned that "most, if not all, blueberry species contain plants that produce functional 2n gametes at low but significant frequencies" (p. 54). The production of 2n gametes in *Vaccinium* is a useful characteristic that helps breeders overcome hybridization barriers caused by differences in the ploidy level in heteroploid crosses. As an example, tetraploid hybrids have been recovered from 4x-2x and 2x-4x crosses due to the production of 2n gametes by the diploid parents (Megalos and Ballington, 1988). The introgression of desirable features from wild diploid genotypes into the cultivated tetraploid germplasm has been possible because of 2n gametes.

The principal objective of this chapter is to survey and compare the pollen fertility of *V. ashei*, *V. constablaei*, *V. corymbosum*, *V. simulatum*, and several F1 hybrid populations originated from them, in order to gather information about their pollen viability, and obtain a chromosome number confirmation for *V. constablaei* and *V. simulatum*. Another objective was to survey and compare those taxa for the presence of unreduced pollen grains, because there is much interest in finding blueberry clones with a high frequency of 2n gamete production.

Materials and Methods

Pollen fertility and the presence of unreduced pollen grains were surveyed in flowers of two *V. constablaei* populations and one *V. simulatum* clone in 1993. Plants from these populations were used as pollen parents in experiments 3 and 4, as described in chapter 2. Branches were cut from plants of these species growing in the wild in Shining Rock Wilderness Area and Roan Mt., near Asheville, NC, on December 23, 1992, and brought to the University of Florida, in Gainesville, FL, in plastic bags in an ice chest containing ice (0°C). In order to provide the flower buds with enough chill units to break dormancy, the branches were stored in a cold room at about 7°C for 30 days. On February 4, 1993, the branches were transferred to a greenhouse for budbreak. Flowering was maintained for several weeks in the greenhouse by immersing the bases of the branches in 1.5 liters of an aqueous preservative solution made of sucrose (5%) + citric acid (5.93×10^{-4}M) + 8-hydroxy quinoline hemisulfate salt (5.93×10^{-4}M). To keep flowering as vigorous and healthy as possible, the bases of the branches were recut and the preservative solution renewed once a week.

In 1994, flowers were collected from three F1 populations of *V. ashei* x *V. ashei* and three F1 populations of *V. ashei* x *V. constablaei* for pollen examination. These populations consisted of 2.3 year-old seedlings growing in a high-density nursery at the Horticulture Unit of the University of Florida in Gainesville. A variable number of well-developed but still unopened flowers was collected at random from four clones of each population. A description of these populations is provided in Table 7.1.

Table 7.1 - Description of the *Vaccinium* seedling populations from which pollen was examined in 1994.

Population	Seed parent	Pollen parent
	(*V. ashei*)	(*V. ashei*)
1	89-176	Powderblue
2	91-54	Premier
3	91-63	Brightwell
	(*V. ashei*)	(*V. constablaei*)
4	85-97	Corvallis # 5
5	85-109	Corvallis # 8
6	91-2	Corvallis # 11

In 1996, flowers from six *V. corymbosum* cultivars (Jubilee, Magnolia, Misty, Sharpblue, Southmoon and Star) were collected in the greenhouse to compare pollen fertility and unreduced gametes with flowers from six *V. corymbosum* x *V. constablaei* F1 clones. The F1 hybrid clones were randomly chosen from a composite of F1 hybrids of four *V. corymbosum* x *V. constablaei* crosses (Florida highbush selections 90-175, 90-178, 90-182 and 93-39 crossed with pollen from *V. constablaei* clones #s 7, 12, 1 and 14, respectively). *Vaccinium constablaei* parents # 1 and 7 were native plants from Shining Rock Wilderness Area near Asheville, North Carolina, and *V. constablaei* parents # 12 and 14 were growing wild on Roan Mt., near the city of Roan Mountain, Tennessee. Branches were cut from those plants, brought to the University of Florida in Gainesville, and when they flowered in the greenhouse, pollen was taken for the pollinations. These crosses were made in 1993 as part of experiment 4, described in chapter 2. Due to the low number of germinated seedlings, in May of 1994 the seedlings were planted as a

composite population in a high-density nursery at the Horticultural Unit of the University of Florida in Gainesville, FL. In March/April, 1996, a variable number of well-developed but still unopened flowers was collected at random from six clones from this population of interspecific hybrids.

The flowers collected were stored in small test tubes or petri dishes in a refrigerator for drying and preservation until the pollen could be examined. Stainability with 2% aceto-carmine glycerol jelly was used as an index of pollen viability. Pollen grains containing cytoplasm are stained with this dye; empty pollen grains are not stained (Radford et al., 1974). Pollen was prepared for examination as follows: first, two drops of 2% aceto-carmine glycerol jelly were placed on a clean slide. Four flowers per sample (clone) were picked at random and a small portion of the apex of the corolla tube, if unopened, was cut off with scissors. Individual flowers were rolled between the indicator finger and the thumb releasing pollen, which was evenly spread onto the aceto-carmine glycerol jelly. The pollen grains immersed in the aceto-carmine glycerol jelly were then covered with a cover slip, and after 15 minutes of staining, the pollen grains were examined under a light microscope. For each sample, one hundred spore units (including tetrads, triads, dyads and monads) were carefully observed, so that the number of stained and unstained pollen grains and the number of unreduced pollen grains that stained could be counted and recorded. Pollen grains that occurred as dyads or monads were considered unreduced. Dyads and monads were carefully examined at a higher magnification under the light microscope to distinguish them from tetrads containing aborted pollen grains.

Pollen fertility, as judged by the percentage of stainable pollen grains, and the percentage of unreduced pollen that stained were estimated for each clone. The data relating to the unreduced pollen were transformed to the root square of (X + 1) prior to the analysis. Differences among taxa on the two pollen characteristics were tested by the analysis of variance according to a completely randomized design and, if significance was achieved, means were compared by the Tukey-test.

Results and Discussion

Pollen Fertility

High frequencies of stainable pollen were found in both *V. constablaei* populations and in the *V. simulatum* clone in 1993, and in *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 hybrids in 1994 (Table 7.2). There were no statistically significant differences among populations in this regard, and the clones shed pollen copiously. The abundance of pollen shed could be an additional indication that their pollen was quite fertile, because aborted pollen tends to be retained inside the anthers according to Stushnoff and Hough (1968), resulting in poor pollen shed.

In 1996, all six *V. corymbosum* x *V. constablaei* F1 clones examined shed pollen poorly and had significantly lower mean frequency of stainable pollen than the six *V. corymbosum* cultivars, all of which shed pollen copiously and showed high frequencies of pollen that stained (Table 7.3). The low pollen fertility indicated that those F1 clones were probably pentaploid hybrids. In the high-density nursery in 1996, these hybrids

Table 7.2 - Frequency of stainable pollen in *V. constablaei* and *V. simulatum* clones in 1993 and in *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 hybrid clones in 1994.

Population	Number of clones examined	Amount of pollen shed	% Stainable pollen grains[Z]	
			Mean	Range
V. ashei x *V. ashei*	12	Copious	96.3[Y]	87.1 - 100.0
F1 (*V. ashei* x *V. const.*)	12	Copious	97.1	87.2 - 100.0
V. const. (Shining Rock)	4	Copious	94.0	84.9 - 99.7
V. const. (Roan Mt.)	4	Copious	96.5	92.7 - 100.0
V. simulatum[X]	1	Copious	84.3	84.3

[Z] One hundred spore units were examined per clone.
[Y] Means within a column followed by different letters are significantly different at $P \leq 0.05$ using the Tukey-test.
[X] The *V. simulatum* clone was not included in the statistical analysis because of the insufficient replication number.

Table 7.3 - Frequency of stainable pollen in *V. corymbosum* and *V. corymbosum* x *V. constablaei* F1 clones in 1996.

Population	Number of clones examined	Amount of pollen shed	% Stainable pollen grains[Z]	
			Mean	Range
V. corymbosum	6	Copious	98.2 a[Y]	92.3 - 100.0
F1 (*V. corymb.* x *V. const.*)	6	Little	48.4 b	3.9 - 63.9

[Z] One hundred spore units were examined per clone.
[Y] Means within a column followed by different letters are significantly different at $P \leq 0.05$ using the Tukey-test.

behaved more like *V. ashei* x *V. constablaei* F1 hybrids than *V. ashei* x *V. ashei* F1 hybrids, in that they flowered late and had a short bloom-to-ripe interval. They appeared

to have a high chill requirement and short fruit development period, features that would have come from *V. constablaei.*

Low pollen fertility in hybrid plants from heteroploid crosses like the *V. corymbosum* x *V. constablaei* hybrids surveyed in 1996 (which probably were pentaploids) could be related to irregularities in chromosome pairing during meiosis, as suggested by Cockerham and Galletta (1976). Pentaploid hybrids from *V. ashei* (6x) crosses with *V. corymbosum* (4x) were investigated by Cockerham and Galletta (1976) and by Vorsa et al. (1987), who reported that they also had low pollen fertility, which ranged from less than 1% to 40%. Other reports of pollen with low stainability were obtained from triploid hybrids originated from *V.corymbosum* (4x) crossed with *V. elliottii* (2x) (Dweikat and Lyrene, 1988) and from *V. corymbosum* triploids (Vorsa and Ballington, 1991). Although there are *V. ashei* and *V. corymbosum* clones with moderate and even poor pollen fertility, these species normally show high frequencies of stainable pollen as shown in this study and in the studies of Cockerham and Galletta (1976), Vorsa et al. (1987), Krebs and Hancock (1990) and Vorsa and Ballington (1991). Pollen from two *V. simulatum* clones was also surveyed by Cockerham and Galletta (1976), who found that one had a high percentage of stainable pollen (94%) and the other a low percentage (16%). These observations, along with our data, indicate that there is wide variation in pollen fertility among *V. simulatum* clones growing in the wild.

The high frequency of stainable pollen in the *V. ashei* (6x) x *V. constablaei* F1 hybrids in 1994 compared to the low frequency of stainable pollen in *V. corymbosum* (4x) x *V. constablaei* F1 hybrids in 1996 support the view that the *V. constablaei* clones used

in this study were hexaploids. This conclusion was also supported by the fact the *V. ashei* x *V. constablaei* crosses gave numerous seedlings per pollinated flower, whereas the *V. corymbosum* x *V. constablaei* crosses gave few.

Unreduced Pollen

Unreduced pollen usually occurs at low frequencies in blueberries. Vorsa and Ortiz (1992) described its formation in a blueberry aneuploid as a result of a combination of events during meiosis, such as the precocious desynapsis of homologous chromosomes at metaphase I due to the absence of crossing over, disjunction of univalent sister centromeres at anaphase I, cytokinesis after telophase I, and the non-occurrence of a second meiotic division. Pollen grains originated from this process usually have the sporophytic chromosome number (2n) and occur in dyads. Monads can also occasionally be formed. These probably have a 4n chromosome number.

In the blueberry taxa surveyed in 1993 and 1994, unreduced pollen was practically non-existent in *V. ashei* x *V. ashei* intraspecific F1 hybrids and in *V. constablaei* clones from Shining Rock Wilderness Area (Table 7.4). On the other hand, low frequencies of unreduced pollen grains were found in *V. ashei* x *V. constablaei* F1 hybrids (in only 2 out of 12 clones), in the only *V. simulatum* clone examined and in the *V. constablaei* clones from Roan Mt. (in 3 out of 4 clones). The frequency of unreduced pollen was significantly higher in the *V. constablaei* clones from Roan Mt. compared to all the other taxa surveyed in 1994, among which there were no statistically significant

differences. In all observed cases, the unreduced pollen was associated with dyads, and they were not always larger than the normal pollen occurring in tetrads.

Table 7.4 - Frequency of stainable, unreduced pollen in *V. constablaei* and *V. simulatum* clones in 1993 and in *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 hybrid clones in 1994.

Population	Number of clones	% Stainable unreduced pollen[z]	
		Mean	Range
V. ashei x *V. ashei*	12	0.00 a[y]	0.00
F1 (*V. ashei* x *V. const.*)	12	0.31 a	0.00 - 3.20
V. const. (Shining Rock)	4	0.00 a	0.00
V. const. (Roan Mt.)	4	1.96 b	0.00 - 4.86
V. simulatum[x]	1	0.55	0.55

[z] One hundred spore units were examined per clone.
[y] Means within a column followed by different letters are significantly different at $P \leq 0.05$ using the Tukey-test.
[x] The *V. simulatum* clone was not included in the statistical analysis because of the insufficient replication number.

Unreduced stainable pollen grains were, however, abundant in the *V. corymbosum* x *V. constablaei* F1 hybrids surveyed in 1996, with a significantly higher mean frequency than in the *V. corymbosum* (highbush) cultivars used as controls (Table 7.5). Unreduced pollen was associated with monads and dyads in the F1 hybrids and only with dyads in the highbush cultivars. Moreover, in the F1 hybrids unreduced pollen grains were larger than normal pollen grains occurring in tetrads but not in the highbush cultivars. These F1 hybrids were presumably pentaploids, in which deviations of the normal meiotic process related to problems in chromosome pairing were expected to occur. Irregularities in

Table 7.5 - Frequency of stainable unreduced pollen in *V. corymbosum* and *V. corymbosum* x *V. constablaei* F1 clones in 1996.

Population	Number of clones	% Stainable unreduced pollen[Z]	
		Mean	Range
V. corymbosum	6	0.62 a[Y]	0.00 - 2.13
F1 (*V. corymb.* x *V. const.*)	6	5.65 b	1.29 - 10.00

[Z] One hundred spore units were examined per clone.
[Y] Means within a column followed by different letters are significantly different at $P \leq 0.05$ using the Tukey-test.

chromosome pairing were probably the principal reason for the production of unreduced pollen grains and aborted pollen. Vorsa and Ortiz (1992) found in a study of blueberry species, that "individuals exhibiting 2n pollen production in the 2x and 4x species typically had lower fertility (percentage of stainable pollen) than those lacking 2n pollen production" (p. 348). The presence of monads, which were noticed only in the pollen of *V. corymbosum* x *V. constablaei* F1 hybrids in 1996, suggests that irregularities in chromosome pairing in these hybrids can sometimes prevent cytokinesis, possibly because of lagging chromosomes during anaphase I. In three triploid hybrids between *V. corymbosum* and *V. elliottii*, Dweikat and Lyrene (1988) reported a range of unreduced pollen from 0.9% to 1.3%, with both dyads and monads present. Vorsa and Ballington (1991) reported that stainable 2n pollen ranged from 0% to 10.3% for *V. corymbosum* triploids.

The observed frequency of unreduced pollen (0% in 1994 for *V. ashei* clones and 0% to 2.1% in 1996 for *V. corymbosum* cultivars) were within the ranges mentioned in the literature for both species, namely from 0% to 11% for *V. ashei* and from 0% to 5.7%

for tetraploid *V. corymbosum* (Cockerham and Galletta, 1976; Megalos and Ballington, 1988; Vorsa and Ballington, 1991; Ortiz et al., 1992a). Evidence for the production of functional 4x gametes from highbush blueberries was reported by Lyrene and Sherman (1983), who obtained pentaploid hybrids from *V. corymbosum* (4x) x *V. elliottii* (2x) crosses.

Vaccinium corymbosum x *V. constablaei* F1 hybrids stood out for their higher production of 2n gametes. These hybrids, presumably pentaploids, can be used as bridges for transferring desirable characteristics from *V. constablaei* into the cultivated highbush blueberries (Jelenkovic and Draper, 1973). A similar approach was suggested by Vorsa et al. (1987) and Laverty and Vorsa (1991) regarding pentaploid hybrids being used for gene transfer between *V. ashei* and the highbush blueberry. Lyrene and Sherman (1985) also suggested that pentaploid highbush x *V. ashei* hybrids can be used as possible cultivars for the U-pick market. Pearl River, a pentaploid (highbush x rabbiteye) F1 hybrid, was recently released as a cultivar in Mississippi (Draper et al., 1994). Therefore, the direct production of cultivars could be another potential use for pentaploid highbush x *V. constablaei* hybrids. Selection of clones for this purpose seems to be feasible, since we observed that some clones have a high fruit set.

This study showed that *V. ashei*, *V. constablaei*, their F1 hybrids, *V. corymbosum* and *V. simulatum* had high pollen fertility. It also showed that *V. corymbosum* x *V. constablaei* F1 hybrids tended to have low pollen fertility. These results indicated that *V. constablaei* is probably hexaploid. Clones with relatively abundant unreduced, stainable pollen grains were found within *V. constablaei* (from Roan Mt.), *V. ashei* x *V.*

constablaei and *V. corymbosum* x *V. constablaei* F1 hybrid populations. Frequency of unreduced gametes in *V. ashei*, *V. constablaei* (from Shining Rock), *V. corymbosum* and *V. simulatum* populations was low to zero.

CHAPTER 8
LEAFING AND FLOWERING

Introduction

Blueberry cultivars vary with respect to the date of vegetative and floral budbreak in the spring. Time of budbreak is affected by the amount of chilling received. Spiers and Draper (1974) and Darnell and Davies (1990), working with rabbiteye blueberries exposed to several chilling periods, observed that flower buds tended to break before or concomitant with vegetative buds, and suggested that this could indicate that flower buds have a lower chilling requirement than vegetative buds. Differences in the time of budbreak between vegetative and flower buds can, however, be attributed to differences in chilling requirement and differences in the number of heat units (GDH) needed to induce growth after the chilling requirement has been satisfied (Lyrene, 1989).

Mainland (1985) reported that in North Carolina, inadequate leaf surface caused by a delay in vegetative bud break in plants that are in full bloom is often a problem and can occur in both highbush and rabbiteye blueberries. He pointed out that warm temperatures during budbreak seemed to cause flower buds to develop faster than leaf buds. When this occurs, leafless branches loaded with developing fruit can die, or the fruit can be small and slow to mature. In Florida, the highbush cultivar Misty tends to produce few leaves until a week or more after full bloom. This delayed leafing weakens

the plants due to the depletion of carbohydrate reserves and predisposes them to dieback caused by stem blight (*Botryosphaeria dothidia*). The high yield of the highbush cultivar Elliot in Michigan has been attributed, in part, to its large total leaf area (Hancock, 1989). This is an additional indication that vigorous early leafing helps to support a high fruit yield in blueberries.

The development of vegetative buds prior to or at about the same time as flower buds is characteristic of highly productive blueberry cultivars growing in regions where they are well-adapted. Therefore, in trying to breed blueberry cultivars that are well-adapted to Florida, strong, early leafing has become an important breeding objective. This chapter discusses efforts to identify sources of early leafing to be used in a breeding program. The leafing and flowering of three taxa (*V. constablaei*, *V. ashei* and *V. ashei* x *V. constablaei* F1 hybrids) at the beginning of the growing season were assessed and compared.

Materials and Methods

Leafing and flowering were first determined in 16 *V. constablaei* clones in the greenhouse on March 3, 1993. These *V. constablaei* clones were the same ones used as pollen parents in experiments 3 and 4 described in chapter 2. Determinations were carried out on branches of those clones that were cut from plants growing wild in Shining Rock Wilderness Area and Roan Mt., near Asheville, North Carolina, on December 23, 1992. The branches were brought to the University of Florida in plastic bags in an ice chest with ice (0°C) and were then stored in the dark at 7°C for 30 days to provide

additional chilling. On February 4, 1993, the branches were transferred to a greenhouse to stimulate budbreak. In the greenhouse, the branches were maintained with their bases immersed in 1.5 liters of an aqueous preservative solution made with sucrose (5%) + citric acid (5.93×10^{-4}M) + 8-hydroxy quinoline hemisulfate salt (5.93×10^{-4}M). Once a week, the bases of the branches were recut and the preservative solution was renewed. Stages of leafing and flowering in the branches were assessed when about 38% of the *V. constablaei* clones had opened flowers.

Leafing and flowering stages were determined in *V. ashei* x *V. constablaei* F1 plants on February 27, 1994, and in *V. ashei* x *V. ashei* F1 plants on March 1, 1994, in a high-density field nursery at the Horticulture Unit of the University of Florida in Gainesville. At that time, the plants were 2.3 years old, and 77.2% of *V. ashei* x *V. ashei* and 2.1% of *V. ashei* x *V. constablaei* F1 plants had opened flowers. Determinations were made on five crosses per type, using 25 to 85 random seedlings from each cross. A description of the crosses is provided in Table 8.1. A second determination was planned for 1994 in the *V. ashei* x *V. constablaei* F1 plants, when about 50% of them had opened flowers. However, a high incidence of blueberry gall midge (*Dasineura oxycoccana*) in that field nursery killed most developing flower buds of *V. ashei* x *V. constablaei* F1 plants, which appeared to be highly susceptible to this insect.

A different set of *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 progenies were assessed for leafing and flowering stages in a high-density field nursery in Gainesville on March 8, 1996. At the evaluation time, the plants were 2.3 years old, and 44% of the *V. ashei* x *V. ashei* and 2% of the *V. ashei* x *V. constablaei* F1 plants had

Table 8.1 - Description of the *V. ashei* x *V. ashei* and *V. ashei* x *V. contablaei* F1 progenies used to assess leafing and flowering stages in 1994.

	Seed parent	Pollen parent
Cross	(*V. ashei*)	(*V. ashei*)
1	85-97	Baldwin
2	91-54	Premier
3	91-60	Brightwell
4	89-317	85-97
5	91-49	W78-69
Cross	(*V. ashei*)	(*V. constablaei*)
6	89-191	NC 86-36-4
7	91-5	Corvallis # 8+10
8	85-97	Corvallis # 8
9	89-178	Corvallis # 8
10	91-2	Corvallis # 11

opened flowers. The *V. ashei* x *V. constablaei* F1 progenies were assessed a second time on April 2, 1996, when 48% of the plants had opened flowers. The selected progenies originated from crosses made in the greenhouse in 1993 as part of experiment 3 (chapter 2). These crosses are described in Table 8.2. Leafing and flowering scores were given to ten plants from each of five crosses per type. The ten plants used from each cross were selected in January, 1996, when they were still completely dormant. The plants selected were those that had the most vigorous canes and the largest number of flower buds in the field. The selected plants were marked for later evaluation.

Leafing stages were scored as follows: 1 = completely dormant; 2 = a few leaves; no leaves fully expanded; 3 = more leaf buds sprouting; very few leaves fully expanded;

4 = many leaves; some fully expanded. Flowering stages were scored as follows: 1 = all flower buds completely dormant; 2 = some flower buds beginning to expand (stages 2 and 3; Spiers, 1978); 3 = no anthesis but flower buds at stages 4 and 5; 4 = one or more flowers at or past anthesis (stages 6 and 7).

Table 8.2 - Description of the *V. ashei* x *V. ashei* and *V. ashei* x *V. contablaei* F1 progenies used to assess leafing and flowering stages in 1996.

	Seed parent	**Pollen parent**
Cross	(*V. ashei*)	(*V. ashei*)
1	91-283	Powderblue
2	90-217	ash 93-25
3	91-287	ash 79-15
4	91-281	ash T105
5	89-186	ash T339
Cross	(*V. ashei*)	(*V. constablaei*)[z]
6	90-217	constablaei # 6
7	90-225	constablaei # 14
8	91-59	constablaei # 8
9	91-59	constablaei # 13
10	91-287	constablaei # 16

[z] *V. constablaei* pollen parents 6, 8 and 16 are wild clones from Shining Rock Wilderness Area, near Asheville, NC; *V. constablaei* clones 13 and 14 are wild plants from Roan Mountain, NC.

Mean leafing and flowering scores were calculated for each type of cross on each evaluation date. Comparisons were made between *V. ashei* x *V. ashei* and *V. ashei* x *V. contablaei* F1 progenies to determine differences in budbreak time. In addition, these two types of progenies were compared to *V. constablaei* clones to study the relative

timing of budbreak for vegetative and flower buds. Means were compared by the Tukey-test when the analysis of variance showed significance due to type of cross.

Results and Discussion

The budbreak of leaves and flowers in the *Vaccinium* taxa studied was almost simultaneous. Although in many clones flower buds tended to break first, leaf budbreak occurred shortly afterward, so that flower and leaf development was overlapping to some extent (Table 8.3). Examining the mean leafing scores for *V. ashei*, *V. constablaei* and their F1 hybrids relative to the stages of flower development, it is evident that leafing increased during the time between initial flower budbreak and full bloom. Mean leafing scores for the F1 (*V. ashei* x *V. constablaei*) hybrids were higher than for *V. ashei* seedlings in the last two stages of flower development, although the differences were not significant by the Tukey-test. *Vaccinium constablaei* clones also tended to have a better mean leafing score than *V. ashei* clones in the last stage of flowering. Nevertheless, *V. constablaei* clones varied widely in this regard. Five out of the 16 clones assessed had poor leafing, whereas eight other clones showed good leafing while in flowering stages 3 and 4. It was also noticed that the *V. constablaei* seedlings, which originated from a composite of open pollinated seeds from 100 clones from Shining Rock Wilderness Area (source 126), used as female parents in experiment 6 in chapter 2, had excellent leafing at the time of flowering in the greenhouse. Chill requirement for these plants had been satisfied during three months in a cold room at 7°C. *Vaccinium constablaei* seedlings from the same source that remained in a high-density field nursery at the Horticultural

Unit of the University of Florida, in Gainesville, FL, showed wide variation in the pattern of leafing and flowering, according to observations made on April 19, 1994. Flowering and leafing were very irregular in some seedlings, probably due to their high chill requirement, which was not satisfied in the field. On April 19, some *V. constablaei* seedlings showed no sign of leaves and flowers, some had good leafing but no open flowers, others tended to flower first and had delayed leafing. Two plants were observed with both good leafing and flowering. Overall, these results and observations suggest that *V. constablaei* clones with good leafing characteristics can be selected and that *V. constablaei* could be used to breed for improved early leafing in *V. ashei* cultivars.

Table 8.3 - Mean leafing score within each flowering score for *V. ashei*, *V. constablaei* and their F1 hybrids[z].

Flowering score	Mean leafing score					
	V. constablaei		F1 (*ash* x *const*)		F1 (*ash* x *ash*)	
	Plants (#)	Mean	Plants (#)	Mean	Plants (#)	Mean
1	0	-	1	2.00	0	-
2	3	2.33[y]	21	3.00	10	3.00
3	7	2.43 b	4	3.75 a	18	3.06 ab
4	6	3.33	24	3.67	22	3.09

[z] Evaluations in *V. constablaei* took place in March, 1993, and in *V. ashei* and F1 hybrids in March, 1996, when approximately 50% of the plants had open flowers. For both flowering and leafing, higher scores indicate a more advanced stage of development (see text).

[y] Means within a row followed by different letters are significantly different at $P \leq 0.05$ using the Tukey-test.

Evaluations of leafing and flowering of *V. ashei* x *V. constablaei* F1 hybrids made when *V. ashei* x *V. ashei* F1 seedlings had open flowers demonstrate that most

interspecific hybrids leafed and flowered later than the *V. ashei* seedlings in both 1994 and 1996 (Tables 8.4 and 8.5). Two percent of the hybrids, however, leafed and flowered

Table 8.4 - Comparison of leafing score means between *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 seedling populations in a field nursery in Gainesville in 1994 and 1996.

F1 populations	Mean leafing score[Z]	
	27 Feb-1 Mar 1994[Y]	8 Mar 1996[X]
V. ashei x *V. ashei*	3.91 a[V]	3.06 a
V. ashei x *V. constablaei*	1.42 b	1.38 b

[Z] Rated on a 4-point scale from 1 (completely dormant) to 4 (very leafy).
[Y] Evaluation in 1994 took place when 77.2% of *V. ashei* x *V. ashei* F1 clones had open flowers.
[X] Evaluation in 1996 took place when 44.0% of *V. ashei* x *V. ashei* F1 clones had open flowers.
[V] Means within a column followed by different letters are significantly different at P≤0.05 through the analysis of variance.

Table 8.5 - Comparison of flowering score means between *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 seedling populations in a field nursery in Gainesville in 1994 and 1996.

F1 clones	Mean flowering score[Z]	
	27 Feb-1 Mar 1994[Y]	8 Mar 1996[X]
V. ashei x *V. ashei*	3.73 a[V]	3.24 a
V. ashei x *V. constablaei*	1.48 b	1.44 b

[Z] Rated on a 4-point scale from 1 (completely dormant) to 4 (very leafy).
[Y] Evaluation in 1994 took place when 77.2% of *V. ashei* x *V. ashei* F1 clones had open flowers.
[X] Evaluation in 1996 took place when 44.0% of *V. ashei* x *V. ashei* F1 clones had open flowers.
[V] Means within a column followed by different letters are significantly different at P≤0.05 through the analysis of variance.

at the same time as *V. ashei* seedlings, showing that there was some variation in these characteristics, which may be genetically determined. The late budbreak of the F1 hybrids is a characteristic inherited from *V. constablaei*, and probably results because the F1 hybrids tend to have a higher chill requirement than the *V. ashei* parents. Late budbreak is a desirable characteristic that would allow the vegetative and flower buds of the F1 hybrids to withstand late freezes that are so common in early March in north-central Florida. Late budbreak due to lack of chilling, however, would be undesirable because it would result in uneven budbreak and a long flowering season. Furthermore, late flowering delays ripening and might increase damage from the blueberry gall midge, whose populations normally increase in north Florida during March.

Leafing relative to flowering stages was observed to be a variable trait in wild *V. constablaei* populations. Nevertheless, F1 (*V. ashei* x *V. constablaei*) hybrids showed better leafing during the more advanced flowering stages than *V. ashei*, this characteristic probably being inherited from *V. constablaei*. Comparison of leafing and flowering score means at a date when *V. ashei* x *V. ashei* seedling populations already had open flowers and developed leaves during two years showed that *V. ashei* x *V. constablaei* F1 seedling populations had a significantly later budbreak.

CHAPTER 9
FLOWERING AND RIPENING TIME

Introduction

The use of *V. constablaei* and *V. simulatum* to incorporate late flowering and short fruit development period, characteristic of these species, into *V. ashei* and highbush blueberry cultivars, respectively, has been investigated as part of the Florida breeding program since 1991. Late flowering cultivars would help the crop avoid late freezes, which are very common in late February and early March in north-central Florida (Lyrene, 1989) and which can kill berries, flowers, and developing flower buds. A short fruit development period would enable late-flowering cultivars to ripen early and could increase the profitability of blueberries produced in Florida and intended for the fresh market.

The best period to harvest blueberries for the fresh market in Florida is April 1 to June 10. After June 10, the quality of the berries falls because of excess rain and high temperatures (Lyrene, 1989). The highest prices are received by growers when blueberries are harvested and shipped between April 1 and May 20. Before April 1, growers face the competition of blueberries imported from the southern hemisphere (Chile and New Zealand), and after May 20, blueberries from North Carolina are brought into the market (Lyrene and Sherman, 1984; Lyrene and Sherman, 1988; Lyrene, 1995).

Early-ripening blueberry cultivars, which provide ripe fruits as early as April (Lyrene, 1987b), constitute an important breeding objective in Florida. One strategy suggested for breeding early-ripening rabbiteye blueberry is to select vigorous, fertile and early-ripening clones from *V. ashei* x *V. constablaei* crosses in the F1 and backcross generations (Lyrene and Sherman, 1984). The cultivar Snowflake, released by the University of Florida breeding program, is an example of the effectiveness of this strategy. Snowflake was produced by backcrossing an F1 *V. constablaei* x *V. ashei* hybrid, created at North Carolina State University, to a low chill Florida *V. ashei* selection and, although it has mostly rabbiteye features, it produces ripe fruits 10 to 14 days before any other rabbiteye cultivar in north-central Florida (Lyrene, 1993).

Southern highbush cultivars trace most of their genes back to *V. corymbosum*, which tends to ripen early. However, southern highbush were bred by hybridizing *V. corymbosum* with later-ripening species, such as *V. darrowi*. Strategies to induce earlier-ripening in southern highbush include crossing *V. corymbosum* with *V. elliottii*, a blueberry native in north Florida and other parts of the southeastern United States. The resulting F1 hybrids lack fruit size, color, and firmness, and these qualities have been hard to recover in later generations (Ballington et al., 1984; Lyrene and Sherman, 1984). *Vaccinium simulatum* is a possible alternative to *V. elliottii* as a source of earliness in crosses with highbush blueberries.

It has been noted that *V. ashei* x *V. constablaei* F1 hybrids tend to show the late-flowering and early fruit ripening typical of *V. constablaei* (Ballington et al., 1986).

Nevertheless, only a few F1 hybrids have been assessed in the field for these two characteristics, and fewer still have been tested in Florida.

Large year-to-year variations in flowering and fruit ripening dates, as well as a large variation among clones has been noticed with blueberries in Florida. Lyrene (1985) reported large variations in flowering and ripening dates among 17 *V. ashei* clones observed for four years in Gainesville, FL. Ballington et al. (1986) assessed several *V. ashei-V. constablaei* derivative progenies in North Carolina in 1984 for several flowering and fruiting characteristics and obtained significant differences among progenies for all traits. They suggested that these differences reflected specific parent combinations and that there was enough variability for selection within progenies. In searching for late-flowering and early-ripening seedlings among these progenies, Ballington et al. (1986) found that only 2% of the seedlings bloomed and ripened at the same time as the late-flowering, early-ripening, highbush cultivar Croatan. This chapter compares flowering and ripening time for *V. ashei* x *V. ashei*, *V. ashei* x *V. constablaei*, *V. corymbosum* x *V. corymbosum* and *V. corymbosum* x *V. simulatum* F1 progenies with *V. constablaei* and *V. simulatum* progenies in Gainesville, FL.

Materials and Methods

Flowering and fruit ripening dates of several *V. ashei* x *V. ashei*, *V. ashei* x *V. constablaei*, *V. corymbosum* x *V. corymbosum*, *V. corymbosum* x *V. simulatum*, *V. constablaei* and *V. simulatum* seedling populations, planted in high-density nurseries at

the Horticultural Unit of the University of Florida, in Gainesville, FL, were recorded in the period of 1993-1996. The populations used differed each year.

Ten *V. ashei* x *V. ashei* and ten *V. ashei* x *V. constablaei* seedling populations from a high-density nursery planted on May 14, 1992, as well as three *V. constablaei* seedling populations planted in another high-density nursery on May 13, 1991, were compared for flowering time in 1993. These progenies and the number of plants used are described in Table 9.1. Plants within each progeny were selected and marked in January, when they were still dormant. The plants selected were those having the most flower buds and the most vigorous canes. From late January through early March, the plants were visited once a week to record the date of the first opened flower. On March 14, a strong freeze with a temperature of -5°C in the field killed all developing flowers, causing the flowering evaluations to be discontinued. Only the flower buds still dormant were not damaged by that freeze. Because the early flowers were killed by the freeze, the ripening time of the various plants was determined, to a greater extent than usual, by the flowering-to-ripening interval. Fruit ripening was assessed twice a week by recording the date of the first ripe berry in every plant of each progeny described in Table 9.1.

Flowering and fruit ripening dates could not be taken from the *V. ashei* x *V. constablaei* F1 populations described in Table 9.1 in 1994 because a high incidence of gall midge (*Dasineura oxycoccana*) killed most developing flower buds in those seedlings. Consequently, six *V. ashei* x *V. constablaei* and eight *V. ashei* x *V. ashei* F1 progenies were selected for evaluation in another nearby high-density nursery, which was

Table 9.1 - Description of the seedling progenies and respective number of plants assessed for flowering and fruit ripening dates in 1993.

Cross	Seed parent	Pollen parent	Number of plants[z]
	(*V. ashei*)	(*V. ashei*)	
1	85-97	Baldwin	10 (20)
2	89-176	Powderblue	10 (71)
3	91-54	Premier	10 (40)
4	91-63	Brightwell	10 (15)
5	91-60	Brightwell	10 (43)
6	89-317	85-97	10 (47)
7	89-310	Premier	10 (50)
8	85-97	84-54 (wild)	10 (74)
9	91-49	W78-69	10 (58)
10	88-151	91-2	10 (39)
	(*V. ashei*)	(*V. constablaei*)	
11	89-191	NC 86-36-4	28 (115)
12	91-5	Corvallis # 8+10	10 (23)
13	85-97	Corvallis # 8	30 (42)
14	89-310	Corvallis # 8	10 (12)
15	85-97	Corvallis # 5	10 (10)
16	89-178	Corvallis # 8	10 (16)
17	91-2	Corvallis # 11	30 (157)
18	85-109	Corvallis # 8	10 (15)
19	89-178	Corvallis # 4	10 (12)
20	90-20	NC 86-36-4	10 (21)
	(*V. constablaei*)	(open pollination)	
23	Corvallis # 1	o.p. at Corvallis	15
24	Corvallis # 2	o.p. at Corvallis	15
25	Corvallis # 3	o.p. at Corvallis	15

[z] The number of plants evaluated for flowering dates is outside parenthesis, and the number of plants evaluated for first ripe fruit dates is inside parenthesis.

planted on May 27, 1993, and in which gall midge was not a problem. This nursery was visited twice a week during the ripening season in 1994 to observe fruit ripening dates. In addition to these progenies, three *V. ashei* x *V. ashei* progeny populations from the previous high-density nursery were included in the evaluations. A description of the progenies used in 1994 is presented in Table 9.2. The date the earliest five seedlings had one or more ripe fruits was recorded for each progeny population.

In 1995, seedlings from five *V. corymbosum* x *V. corymbosum* and five *V. corymbosum* x *V. simulatum* F1 progenies, as well as one *V. constablaei* and one *V. simulatum* progeny, were marked in the high-density nursery planted on May 27, 1993, to record flowering and fruit ripening dates. The *V. constablaei* progenies originated from open-pollinated seed collected from wild plants growing in Shining Rock Wilderness Area (near Asheville, NC), and the *V. simulatum* progenies came from open-pollinated seed collected on Grandfather Mt. (near Linville, NC). Seedlings from five *V. ashei* x *V. ashei* and four *V. ashei* x *V. constablaei* F1 progenies growing in a high-density nursery planted on May 30, 1994, were also selected and marked for evaluation. These seedlings originated from crosses made in the greenhouse in March, 1993, as part of experiment 3 described in chapter 2. The parents of the progenies are listed in Tables 9.3 and 9.4. Ten plants were selected and marked in each progeny. Plants with the most vigorous canes and the most flower buds within each progeny were chosen. Plants were visited once a week, and the dates they showed one or more open flowers and one or more ripe fruits were recorded.

Table 9.2. - Description of the seedling progenies assessed for fruit ripening in 1994.

Cross	Seed parent	Pollen parent
	(*V. ashei*)	(*V. ashei*)
1	89-176	81-273
2	89-314	85-97
3	92-55	92-43
4	89-314	91-58
5	92-61	85-111
6	92-62	Snowflake
7	90-18	92-40
8	89-197	92-151
9	85-97	Baldwin
10	91-54	Premier
11	91-60	Brightwell
	(*V. ashei*)	(*V. constablaei*)[z]
12	Snowflake	91-362 (Corvallis)
13	Snowflake	Composite of 5 Corvallis const.
14	Windy	92-160 (Corvallis)
15	88-195	92-284 (Corvallis)
16	89-177	91-364 (Corvallis)
17	83-94	92-285 (Corvallis)

[z] *V. constablaei* seedlings selected in field nurseries in Gainesville, Florida. The seedlings were grown from open-pollinated seed harvested from field plots of five *V. constablaei* clones growing in the USDA germplasm repository, Corvallis, Oregon.

The same *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* seedling populations that were evaluated in 1995 were again used to assess flowering and fruit ripening dates in 1996. One additional *V. ashei* x *V. constablaei* population was included. Plants were reselected within the populations, and the ten plants with the most vigorous canes and the

most numerous flower buds were selected for observation in each population. Plants were examined twice a week to record for each plant the dates when the first flower opened and the first berry ripened.

Table 9.3. - Description of the *V. corymbosum* x *V. corymbosum*, *V. corymbosum* x *V. simulatum* and *V. simulatum* progenies assessed for flowering and fruit ripening in 1995.

Progeny	Seed parent	Pollen parent
	(*V. corymbosum*)	(*V. corymbosum*)
1	91-16	87-217
2	82-217	81-83
3	92-79	91-156
4	92-97	6-19
5	92-81	90-171
	(*V. corymbosum*)	(*V. simulatum*)[Y]
6	HB cvs[Z]	92-286
7	Marimba	92-156 + 92-152 B
8	90-174	92-152 C
9	90-173	92-156
10	90-150	92-157
	(*V. simulatum*)[X]	(*V. simulatum*)[X]
11	Grandfather Mt.	open-pollination

[Z] Composite of 6 crosses.
[Y] All originated from Grandfather Mt., North Carolina.
[X] *V. simulatum* seedlings grown from a composite of open-pollinated seeds from 30 plants from Grandfather Mt.

Table 9.4. - Description of the *V. ashei* x *V. ashei*, *V. ashei* x *V. constablaei* and *V. constablaei* progenies assessed for flowering and fruit ripening in 1995 and 1996.

Progeny	Seed parent	Pollen parent
	(*V. ashei*)	(*V. ashei*)
1	91-283	Powderblue
2	90-217	93-25
3	91-287	79-15
4	91-281	T105
5	89-186	T339
	(*V. ashei*)	(*V. constablaei*)[Z]
6	90-217	constablaei # 6
7	91-59	constablaei # 13
8	91-59	constablaei # 8
9	91-287	constablaei # 16
10[Y]	90-225	constablaei # 14
	(*V. constablaei*)[X]	(*V. constablaei*)
11	Shining Rock Wilderness Area	open-pollination

[Z] All *V. constablaei* pollen parents were wild clones selected from the high mountain balds (about 2000 m) of western North Carolina or eastern Tennessee. Plants 6, 8 and 16 were from the Shining Rock Wilderness Area, near Asheville, NC. Plants 13 and 14 were from Roan Mt., NC.

[Y] This progeny was evaluated only in 1996.

[X] *V. constablaei* seedlings originated from a composite of open-pollinated seeds from 100 clones from Shining Rock Wilderness Area, evaluated only in 1995.

Results and Discussion

Flowering Time

Large variations in flowering time were observed among the taxa studied from 1993 to 1996. *Vaccinium ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 hybrids

started to flower almost simultaneously in late January,1993. Flowering continued at a slow pace through February and early March of 1993. On March 14, a strong freeze killed all developing flowers. At the last evaluation date (March 7), about one third of the *V. ashei* plants still had no open flowers compared to two thirds of the *V. ashei* x *V. constablaei* hybrids and the whole *V. constablaei* population (Fig. 9.1). This observation suggests a much higher chill requirement for *V. constablaei* populations compared to *V. ashei* populations and an intermediate chill requirement for *V. ashei* x *V. constablaei* F1 hybrids. The mid-winter flowering of *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 hybrids was unusual and unexpected and can probably be related to the warm temperatures in January (Table 9.5). According to Lyrene and Sherman (1985), average temperatures for December, January and February are good indicators of how much chilling blueberry plants receive in the field. It is doubtful that the chill requirement had been completely satisfied in both types of hybrids when flowering started in late January. However, it is not unusual in Florida for under-chilled blueberry plants to open a few flowers early in the season.

Flowering time was not directly assessed in 1994. Nevertheless, *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 hybrid progenies from the same high-density nursery used the previous year were scored for leafing and flowering stages in 1994, as described in chapter 8. The data were taken on February 27 - March 1, when 77.2% of the *V. ashei* plants already had one or more opened flowers. At this time, the *V. ashei* x *V. constablaei* F1 hybrid plants were still quite dormant, indicating that they flowered significantly later than the *V. ashei* progenies. Temperatures were lower in December

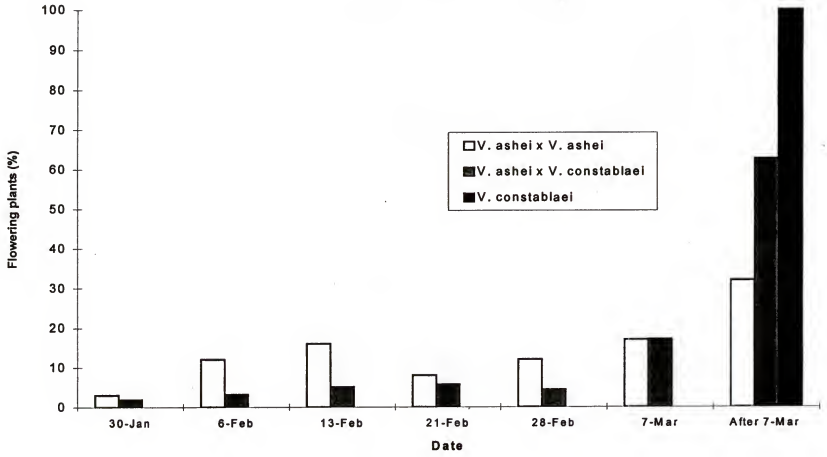

Fig 9.1 - Date of first open flower of *V. ashei* x *V. ashei* (based on 100 plants), *V. ashei* x *V. constablaei* (158 plants) and *V. constablaei* (45 plants) in 1993.

1993 and January 1994 than in February 1994 (Table 9.5), which contributed to the accumulation of chill units. Higher temperatures in February stimulated earlier flowering in the *V. ashei* plants than in the *V. ashei* x *V. constablaei* F1 hybrid plants, which had a higher chill requirement.

Table 9.5. - Mean temperature during the winter months in Gainesville, FL, in the period of 1993 - 1996.

Winter	Mean temperature (°C)[Z]			
	December	January	February	Average
1992/93	13.9	15.8	12.1	13.9
1993/94	10.5	11.6	16.1	12.7
1994/95	14.4	11.4	12.8	12.9
1995/96	12.0	11.3	13.4	12.2
Normal (1951-1980)[Y]	13.6	12.3	13.4	13.1

[Z] Temperatures were taken from a meteorological station in Gainesville (11 WNW), cited by NOAA (1992, 1993, 1994, 1995, 1996).
[Y] Normal temperatures were taken from the Gainesville municipal airport, cited by NOAA (1992).

In 1995, *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 hybrids again flowered simultaneously, mainly during March (Fig. 9.2). Probably, the lower temperatures in January and February provided enough chilling to permit both types of hybrids to flower as soon as temperatures warmed in late February and early March. *Vaccinium constablaei* plants, however, flowered sporadically and much later, during April and May; their much higher chill requirement was not satisfied under Gainesville field conditions.

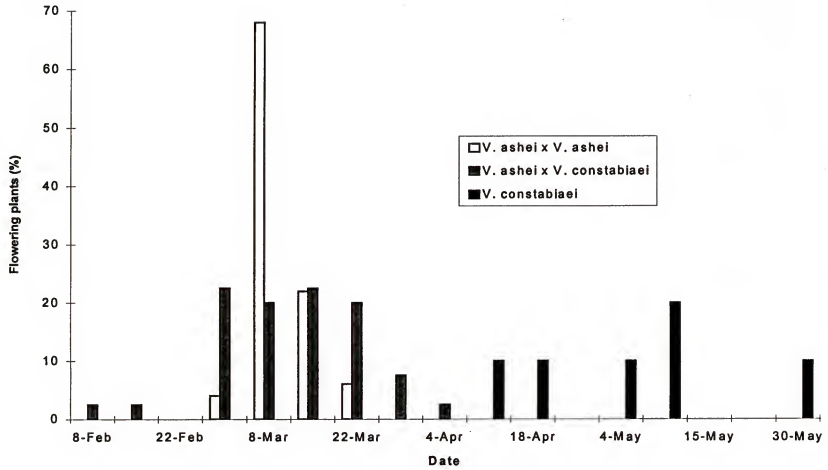

Fig 9.2 - Date of first open flower of *V. ashei* x *V. ashei*, *V. ashei* x *V. constablaei* and *V. constablaei* plants in 1995 (based on 50, 40 and 10 plants of each type, respectively).

Vaccinium corymbosum x *V. simulatum* F1 hybrids were also assessed in 1995. Compared to *V. corymbosum* x *V. corymbosum* F1 hybrids, they flowered shortly afterward, mainly in March, possibly because of a higher chill requirement inherited from *V. simulatum* (Fig. 9.3). Like *V. constablaei*, *V. simulatum* plants flowered sporadically and much later in the season, indicating that their chill requirement was not satisfied in Gainesville. The *V. corymbosum* x *V. corymbosum* F1 plants flowered in early February in 1995, which was the earliest of all the taxa studied. Early flowering makes them very susceptible to damage from late freezes. On the other hand, they are well adapted to north-central Florida in that they have a low chill requirement and have been developed to produce ripe blueberries for the fresh market in April and early May, when prices are high.

In 1996, as in 1994, most *V. ashei* x *V. constablaei* F1 plants started flowering later than *V. ashei* plants (Fig. 9.4). The temperatures of December and January probably were not cold enough to satisfy the higher chill requirement of *V. ashei* x *V. constablaei* F1 plants but were enough to break dormancy of flower buds of *V. ashei* x *V. ashei* F1 plants. Rising temperatures during February favored flowering of *V. ashei* plants before *V. ashei* x *V. constablaei* F1 plants.

Our observations confirmed that *Vaccinium ashei* and *V. constablaei* have very distinct chill requirements. While *V. ashei* cultivars have a chill requirement of 300 to 600 hours below 7.2°C (Darnell et al., 1992), *V. constablaei* clones require more than 1500 hours (Galletta, 1975; Lyrene, 1987a). *Vaccinium ashei* x *V. constablaei* F1 hybrids flowered shortly after *V. ashei* plants in 1994 and 1996. The most likely explanation for

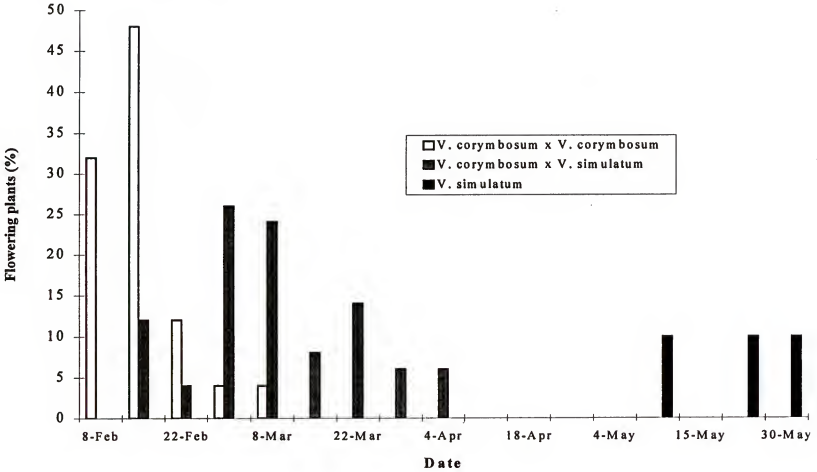

Fig 9.3 - Date of first open flower of *V. corymbosum* x *V. corymbosum*, *V. corymbosum* x *V. simulatum* and *V. simulatum* plants in 1995 (based on 50 plants of each type except 10 for *V. simulatum*).

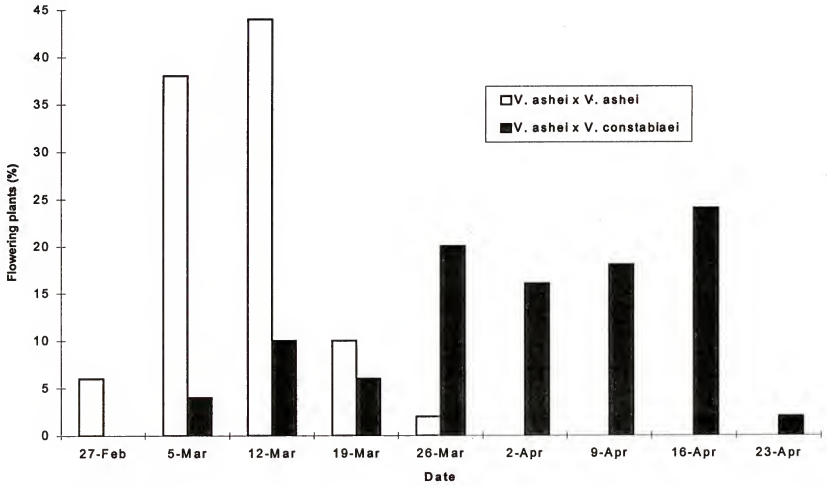

Fig 9.4 - Date of first open flower of *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* plants in 1996 (based on 50 plants of each type).

this is that the hybrids have a chill requirement intermediate between the parents but much closer to *V. ashei* than to *V. constablaei*. The method used to score flowering dates for the plants in this study, which was to record the date when the first flowers opened on each plant, probably accounted in part for the apparent dominance of the low-chill phenotype. One symptom of an under-chilled plant is an extended flowering period, with different flower buds on the same plant breaking dormancy over a period of several weeks. Since the earliest buds that broke dormancy were used to classify the plants in this study, their chill requirement probably appeared lower than if the date of 50% flowering or 80% flowering had been the basis for comparing the taxa. *Vaccinium constablaei* has such a high chill requirement that some clones flowered very late in the season and others did not even flower in the field conditions of Gainesville, FL, even though flower buds had been formed the previous autumn. *Vaccinium simulatum*, like *V. constablaei*, has a very high chill requirement and can be a source for late flowering in crosses with *V. corymbosum*. The seedling observations indicate that the mode of gene action was partial dominance of *V. ashei* and *V. corymbosum* genes for chill requirement over *V. constablaei* and *V. simulatum* genes in the F1 hybrids, respectively.

In addition to the differences in flowering time observed among the taxa studied, large variations were found among clones within each taxon due to segregation. These variations among individual clones are primarily attributed to genotypic differences in chill requirement and also in heat units required to make the flowers open after chilling is satisfied, and provide many possibilities for selection. Variations in flowering time between years for each taxon are attributed to environmental factors, especially

temperature. Selection based on flowering dates still can be effective despite the variation among years. Lyrene (1983a) reported that the repeatability (seedling correlation) for flowering dates of 54 rabbiteye seedlings over two years was 0.61, which is fairly high.

Fruit Ripening Time

The time of flowering and the flowering-to-ripening interval were both major factors in determining the ripening dates of the taxa studied. Comparisons of fruit ripening time between *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* F1 hybrids showed a small difference between them, primarily because the *V. ashei* seedlings tended to flower earlier and the *V. ashei* x *V. constablaei* F1 plants tended to have a shorter fruit development period, a characteristic from *V. constablaei*. Some *V. ashei* x *V. constablaei* F1 seedlings had ripe fruits one week before the earliest *V. ashei* x *V. ashei* F1 seedlings in 1993 (Fig. 9.5). The earliest fruit from both groups presumably developed from flowers that opened after the March 14 freeze. This freeze killed the early flowers on both types of seedlings. A large variation, due to segregation, was found among seedlings within each of the two types regarding the date the first berry ripened, which ranged from May 13 to July 13.

Vaccinium ashei x *V. ashei* F1 seedlings started ripening somewhat earlier than *V. ashei* x *V. constablaei* F1 seedlings in 1994 (Fig. 9.6). This probably happened because *V. ashei* plants flowered significantly earlier but had a longer fruit development period than *V. ashei* x *V. constablaei* F1 plants in that year. Differences in the time of flowering

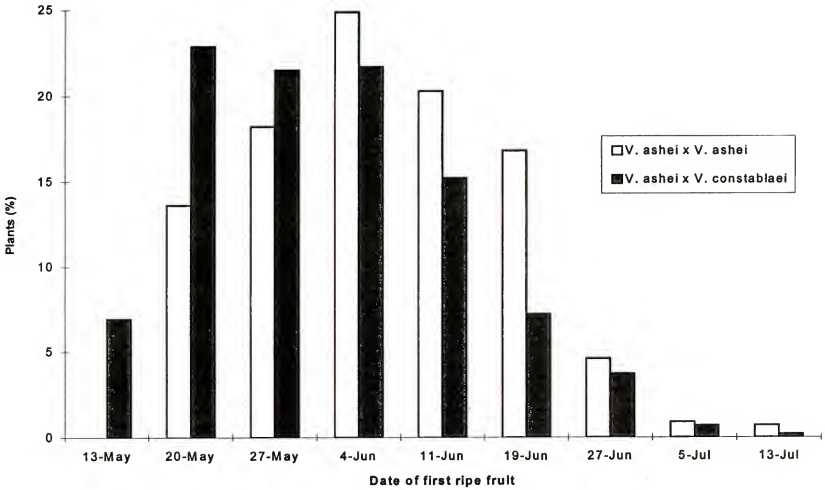

Fig 9.5 - Percentage of the seedlings of two types that produced their first ripe fruit on various dates in 1993.

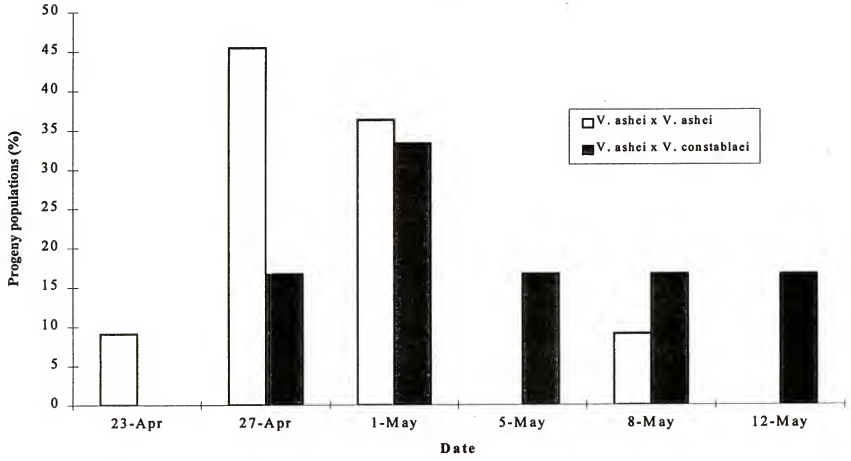

Fig 9.6 - Percentage of the progeny populations of two types in which the earliest 5 seedlings had one or more ripe fruit by various dates in 1994 (based on a total of 11 *V. ashei* x *V. ashei* and 6 *V. ashei* x *V. constablaei* populations, each population with a number of plants that varied from 55 to 125 plants).

appeared to have been, at least in part, compensated by differences in the fruit development period between the two types of hybrids.

In 1995, *V. ashei* x *V. constablaei* F1 seedlings started having ripe fruits two weeks before *V. ashei* x *V. ashei* F1 seedlings (Fig. 9.7). In that year, flowering occurred at about the same time for both types of hybrids. These observations confirmed that *V. ashei* x *V. constablaei* F1 seedlings had a shorter fruit development period than *V. ashei* seedlings. Only one *V. constablaei* plant produced fruit in the field in 1995. This fruit was late, ripening in May, after most seedlings of the other two types already had ripe fruits. Although *V. constablaei* has a short fruit development period, late flowering due to lack of chilling resulted in late fruit ripening.

Fruit ripening time for *V. corymbosum* x *V. corymbosum* and *V. corymbosum* x *V. simulatum* F1 seedlings showed some overlap in 1995 (Fig. 9.8). However, 92% of the *V. corymbosum* seedlings had ripe fruit in April compared to 34% of the *V. corymbosum* x *V. simulatum* seedlings. Although *V. corymbosum* x *V. simulatum* seedlings appeared to have a short fruit development period, their late flowering in relation to *V. corymbosum* seedlings in 1995 resulted in the late ripening of most of the seedlings. *Vaccinium simulatum* plants produced no fruit in 1995; most did not flower; the rest produced a few flowers late in the season. The fruit development period of *V. corymbosum* x *V. simulatum* seedlings did not seem to be shorter than the fruit development period of *V. corymbosum* seedlings (Fig. 9.3 and Fig. 9.8). A possible explanation is that *V. corymbosum* has been under recurrent selection in Florida for early fruit ripening for several generations, which may have reduced the fruit development period.

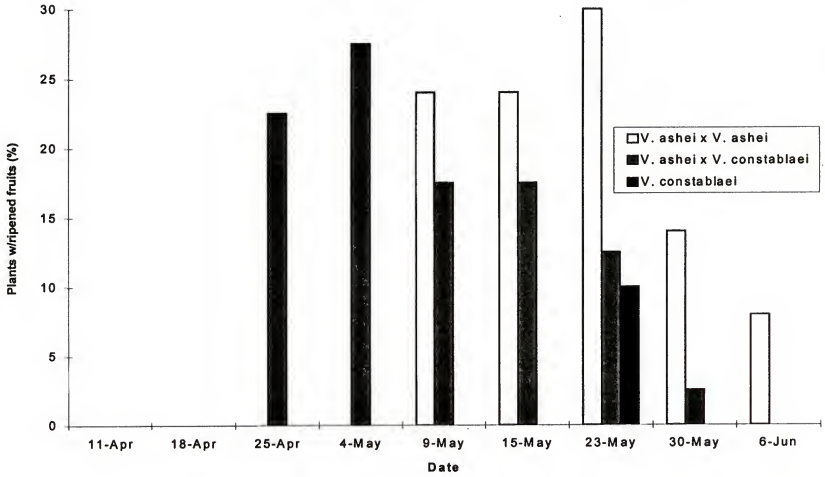

Fig 9.7 - Date of first ripe fruit of *V. ashei* x *V. ashei*, *V. ashei* x *V. constablaei* and *V. constablaei* plants in 1995 (based on 50, 40 and 10 plants of each type, respectively).

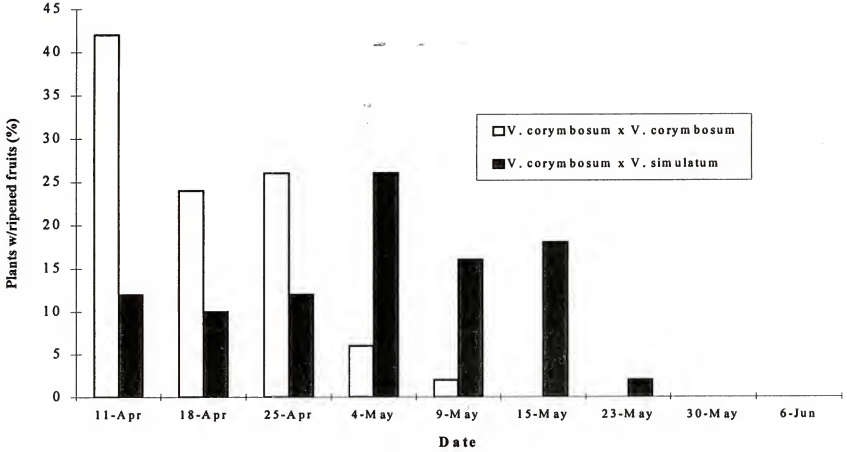

Fig 9.8 - Date of first ripe fruit of *V. corymbosum* x *V. corymbosum*, *V. corymbosum* x *V. simulatum* and *V. simulatum* plants in 1995 (based on 50 plants of each type).

Vaccinium ashei x *V. ashei* and *V. ashei* x *V. constablaei* F1 seedlings ripened at about the same time in 1996, when, like in 1994, differences in the flowering time were compensated by differences in the fruit development period between the two types of hybrids (Fig. 9.9).

Our observations show that it is possible to select *V. ashei* x *V. constablaei* and *V. corymbosum* x *V. simulatum* F1 clones with early ripe fruits, because there is much variation among seedlings. High repeatability for ripening dates of rabbiteye seedlings between years ($r = 0.84$) found by Lyrene (1983a) indicates that much variation in ripening dates is genetic, and selection for early ripening can be effective. In colder areas, where the chilling requirement of the interspecific hybrids is fully satisfied, they could show even more earliness (compared to *V. corymbosum* and *V. ashei*) than they did in Gainesville.

The results indicated that the late flowering and short bloom-to-ripen period, typical of *V. constablaei* and *V. simulatum*, are characteristics present in the *V. ashei* x *V. constablaei* and *V. corymbosum* x *V. simulatum* F1 hybrids. Late flowering, however, was much dependent on the mean temperatures of the winter months (December, January and February). Both flowering time and fruit development period had a great influence on the fruit ripening time.

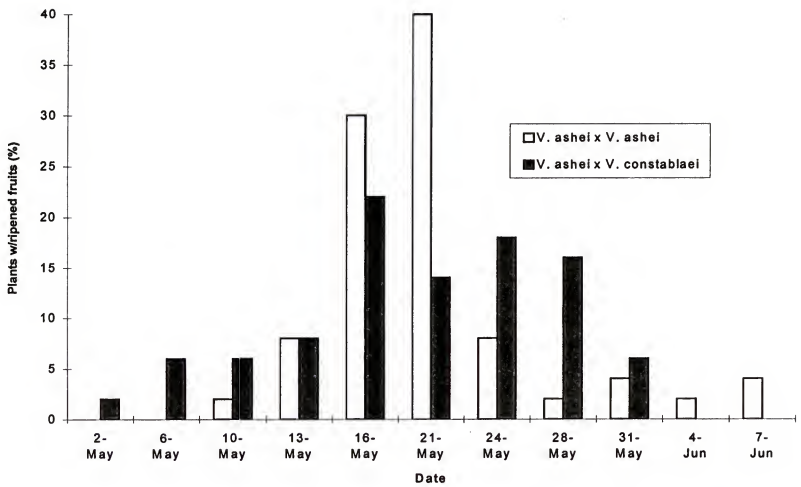

Fig 9.9 - Date of first ripe fruit of *V. ashei* x *V. ashei* and *V. ashei* x *V. constablaei* plants in 1996 (based on 50 plants of each type).

CHAPTER 10
CONCLUSIONS

This research shows that *V. ashei* x *V. constablaei* and *V. corymbosum* x *V. simulatum* crosses are viable and produce fertile F1 progeny. Fertility is not expected to be a limitation in using *V. constablaei* and *V. simulatum* to improve rabbiteye (*V. ashei*) and highbush (*V. corymbosum*) blueberries, respectively, in Florida. *Vaccinium constablaei* can contribute genes to the rabbiteye blueberry for better leafing, later flowering, short bloom-to-ripe period, flower features that are more favorable to pollination by honeybees, and higher self fertility. *Vaccinium simulatum* can contribute genes for late flowering and short bloom-to-ripe period to southern highbush blueberries.

Large variability in all studied traits, including fruit quality traits, in both *V. ashei* x *V. constablaei* and *V. corymbosum* x *V. simulatum* hybrids makes selection for desirable characteristics possible in the F1 generation. However, development of acceptable commercial rabbiteye and highbush cultivars for north-central Florida that contain *V. constablaei* and *V. simulatum* genes, respectively, will probably require testing many parental combinations and screening large populations of seedlings.

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BIOGRAPHICAL SKETCH

Rogério Ritzinger was born in Porto Alegre, Rio Grande do Sul, Brazil. He attended elementary, middle and high school there. In 1976, he entered the Federal University of Rio Grande do Sul, from which he received a Bachelor of Science degree in agronomy in 1980. After graduation, he continued his education at the Federal University of Rio Grande do Sul pursuing a Master of Science degree in plant science. He obtained the degree in 1984. His research dealt with testing plant spacings for the yellow passion fruit (*Passiflora edulis* var. *flavicarpa*). In 1985, he was hired by EMBRAPA (a brazilian agricultural research agency) as a fruit crop researcher. Most of his work was related to the evaluation and characterization of clones and varieties of tropical and sub-tropical fruit species, including citrus, guarana (*Paullinia cupana* var. *sorbilis*) and pineapple, for the State of Acre. In 1992, he was accepted by the Horticultural Sciences Department of the University of Florida as a graduate student in a PhD program in plant breeding. He was awarded a scholarship from EMBRAPA for this purpose. After the completion of the requirements of the PhD program, he intends to resume his work with EMBRAPA as a fruit crop breeder.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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